THE PERSISTENCE OF POLYNUCLEAR AROMATIC HYDROCARBONS IN SOIL

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ABSTRACT

This report reviews information regarding the sources and properties of PAH's and processes affecting their persistence when disposed of in soil. It presents the results of incubation studies conducted to further define the fate of PAH's added to an unacclimated agricultural soil.

Two incubation studies were performed. In the first, a mixture of eight PAH's was added to soil and the concentrations of each compound were monitored with time. In the second, ^{14}C labelled benzo(a)pyrene and anthracene were added to soil in biometer flasks. The distribution of ^{14}C as volatile, adsorbed and degraded products was determined in sterilized and biologically active soil.

The kinetics of disappearance were defined for several PAH's, with reaction orders ranging between 0 and 2, depending on the compound. In some cases, two kinetic stages were observed. Greater persistence was generally associated with PAH's of high molecular weight and sorptivity and low water solubility. The low to medium molecular weight PAH's were reduced in concentration by 94 to 100% in 200 days, while the high molecular weight PAH's were reduced by 22 to 88% in 400 days. Mechanisms leading to a decrease in PAH concentrations were predominantly volatilization and adsorption to soil solids for anthracene, and adsorption to soil solids for benzo(a)pyrene. Microbial transformation of benzo(a)pyrene was minimal.

The results of this study are specific to unacclimated agricultural soil incubated under specified conditions. The relevance of these results to land treatment of oily wastes is discussed and recommendations made for waste and soil monitoring programs and for future research.

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1 EXECUTIVE SUMMARY

Polynuclear aromatic hydrocarbons (PAH's) are a group of organic chemicals which are produced by a variety of industrial processes, including the refining of oil, and which have been identified as potential environmental and health hazards. This report reviews information regarding the sources and properties of PAH's and processes affecting their persistence in soil, and describes studies to further define the fate of PAH's in soil.

A review of the literature indicated that industrial activity has resulted in inputs of PAH's to the environment in excess of natural inputs. Some PAH's are toxic, carcinogenic and/or mutagenic and because of their low water solubility and high lipid solubility may persist in the environment and bioaccumulate. Reports have indicated that the higher molecular weight PAH's tend to persist in soil and sediments and bioaccumulate in aquatic organisms.

Processes that may affect the persistence of PAH's in soil include adsorption to soil solids, volatilization, photochemical degradation, microbial degradation and leaching. Two incubation studies were performed to assess the effects of the above processes on PAH loss from soil. In the first, a mixture of eight PAH's [naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene and benzo(a)pyrene] was added to soil at levels of 5 and 50 $mg \cdot kg^{-1}$ and the concentration of each compound was monitored with time. In the second, ^{14}C labelled benzo(a)pyrene and anthracene were added to soil in biometer flasks. The distribution of ^{14}C as volatile, adsorbed and degraded products was determined in sterilized and biologically active soil. These studies were performed using unacclimated agricultural soil as a first step in identifying the fate of PAH's in soil.

The kinetics of disappearance from soil were defined for several PAH's added to soil as pure compounds. For some PAH's, however, little change in concentration occurred within the incubation period at the 5 $mg \cdot kg^{-1}$ level of PAH addition and discrimination between kinetic models was not possible. Reaction orders that were identified ranged between 0 and 2, depending on the PAH compound. The use of a first order model to predict disappearance from soil, as is often performed, is clearly not adequate for some PAH's and if used will severely underestimate their persistence in soil.

Naphthalene, phenanthrene, anthracene, pyrene and fluoranthene initially disappeared rapidly from soil during an initial period of 200 days or less. A loss of 94 to 98% occurred during this initial period and approximated first order kinetics, in some cases following a lag period. Within the initial period, with the exception of anthracene, the first order kinetic rate constants were the same for 5 and 50 $mg \cdot kg^{-1}$ additions of PAH. Following the initial period, the remaining 2-6% of the added PAH was lost at a much reduced rate and the first order rate constants tended to be higher with the 50 $mg \cdot kg^{-1}$ addition than the 5 $mg \cdot kg^{-1}$ addition of PAH.

Losses of only 22 to 88% were observed for benzo(a)anthracene, chrysene and benzo(a)pyrene and only one kinetic period was identified

within the 400-day incubation period. With chrysene the first order kinetic rate constants were the same at the 5 and 50 $mg \cdot kg^{-1}$ levels of addition, however, for benzo(a)anthracene and benzo(a)pyrene the rate constants differed. The disappearance of benzo(a)anthracene approximated first order kinetics, however a zero order model was generally appropriate for the disappearance of benzo(a)pyrene and chrysene.

The mechanisms of disappearance of anthracene and benzo(a)pyrene were assessed using ^{14}C labelling. The results indicated that biological activity was responsible for some of the loss of anthracene from soil. Binding to soil solids and volatilization (either as anthracene or as metabolites) were identified as the major loss mechanisms. Identification of loss mechanisms for benzo(a)pyrene was less successful due to the small amount of benzo(a)pyrene that reacted within the incubation period. Binding of benzo(a)pyrene to soil solids appeared to be the major mechanism involved, while microbial transformation of the compound was minimal. At this time, little is known about the significance of soil bound PAH's in the environment.

The information from this study generally agrees with observations in the literature and provides more detailed information on the disappearance kinetics and mechanisms than was previously available for some PAH's. Greater persistance was associated with PAH's of high molecular weight and sorptivity and low water solubility. The results of this study are specific for the addition of pure PAH's added to Donnybrook sandy loam. Different rates of PAH disappearance may be observed with different environmental matrices, soil types and field conditions. The results do suggest that, depending on concentration in waste and on application rate and frequency, accumulation of low and intermediate molecular weight PAH's may occur on land treatment sites. Further incubation studies have been planned to evaluate the persistence of PAH's in oil refinery waste applied to soil.

On the basis of this study the following recommendations are made:

1. That those responsible for the disposal of oily wastes to land be made aware that certain PAH's persist and may accumulate in soil. It is desirable that analysis of PAH's be added to soil monitoring programs to assess accumulation on individual sites.

2. That long term studies be performed to assess the environmental impact, including the effect on groundwater quality and bioavailability, of PAH's added to different soil and waste types and under a variety of environmental conditions.

3. That the identity and fate of soil bound PAH's and metabolites should be determined.

4. That studies be undertaken to identify waste management techniques which enhance PAH degradation or removal from soil.

2 INTRODUCTION

Polynuclear aromatic hydrocarbons (PAH's) are organic chemicals consisting of fused benzene rings. They may exist as unsubstituted nuclear structures or with alkyl substituents and are part of a larger group of organic chemicals, polynuclear aromatics (PNA's) which are similar in their properties and are currently of environmental concern (Sims and Overcash, 1983). PNA's include fused ring structures in which carbon has been replaced by nitrogen, sulfur or oxygen to form heteroaromatic compounds. PAH'S, and other PNA's, are formed in a variety of industrial processes, particularly those which involve pyrolysis of organic molecules, as in coke production and oil refining, and wastes from these industries release appreciable amounts of PAH's into the environment. The fate of PAH's in the environment is not well defined but is of concern because many show the potential of being toxic, carcinogenic and/or mutagenic and they may bioaccumulate or persist in the environment.

This study investigated the persistence of eight unsubstituted PAH's (naphthalene, phenanthrene, anthracene, pyrene, fluoranthene, benzo(a)anthracene, benzo(a)pyrene and chrysene) in soil. Microbial degradation, sorption, volatilization and photochemical degradation were assessed as fate mechanisms through the use of ^{14}C labelled anthracene and benzo(a)pyrene. Although specific for PAH addition to unacclimated agricultural soil, this study is the first in a series of studies to identify the persistence and fate of PAH's in industrial waste applied to land. This information should facilitate the development of recommendations for disposal of wastes containing PAH's.

3 BACKGROUND INFORMATION

3.1 Structures and Properties

The chemical structures of the PAH's studied are presented in Figure 3.1, and range from the two-ring, low molecular weight naphthalene to five-ring, high molecular weight benzo(a)pyrene. The different number of rings and the orientation of the rings result in differences in the environmental properties of PAH's. Selected environmental properties are listed in Tables 3.1.1 and 3.1.2 for the eight PAH's studied.

Many PAH's are toxic, carcinogenic, and/or mutagenic and possess chemical properties that indicate they are persistent in the environment and tend to bioaccumulate. The relative toxicities and bioaccumulation potential are presented in Table 3.1.1. There is a general tendency for acute toxicity to occur with the low molecular weight, relatively water soluble PAH's, such as naphthalene, while subacute effects such as carcinogenicity and bioaccumulation tend to occur with the high molecular weight, less soluble PAH's. The implications of low water solubility and high lipid solubility include: (a) the production of cytotoxins through efficient microsomal metabolism in cells and (b) long term storage in fat deposits leading to chronic exposure and bioaccumulation (Radding et al, 1976). Detailed reviews of the toxic, mutagenic and carcinogenic effects of PAH's are available in a publication by the International Agency for Research on Cancer (IARC, 1973) and the review by Santodonato et al (1981).

The data presented in Table 3.1.2 indicate that volatility (vapour pressure) is high for low molecular weight PAH's but lipid solubility (log octanol/water coefficient) and sorptivity to solids (sorption coefficient) is high for the high molecular weight PAH's. These properties affect the partitioning of PAH's between aqueous, gaseous and solid phases in an environmental matrix and thus will affect the fate of PAH's in a soil system.

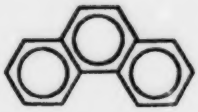
3.2 Sources

PAH's occur naturally in the environment and as a result of industrial activities. Natural sources include the products from forest and grass fires, geochemical processes and synthesis by micoorganisms and plants. Background concentrations in environmental materials (presumed to result from natural sources) range from 0.01 to 88 $\mu g \cdot kg^{-1}$ in soil (Sims and Overcash, 1983), .001 to 0.1 $\mu g \cdot L^{-1}$ in groundwater (Radding et al, 1976) and 1 to 10 $\mu g \cdot kg^{-1}$ in plants (Edwards, 1983).

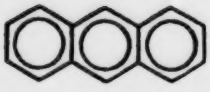
Certain industrial activities which involve pyrolysis of organic molecules result in substantial contributions of PAH's to the atmosphere and soil. The American Petroleum Institute reported that the majority of benzo(a)pyrene in the atmosphere is produced by thermal generating plants, coke production and open burning (Brown and Weiss, 1982). PAH's discharged to the atmosphere reach soil or water as particulate fallout. PAH's may also reach soil through spillage or disposal of wastes. It was reported (Brown and Weiss, 1982) that concentrations of 0.2 to 0.5 $mg \cdot kg^{-1}$ of benzo(a)pyrene were found in untreated petroleum refinery wastewater, and

FIGURE 3.1 CHEMICAL STRUCTURES OF THE PAH'S STUDIED

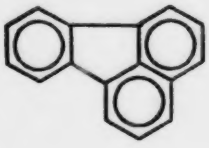
Naphthalene



Phenanthrene

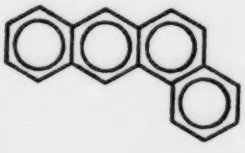


Anthracene

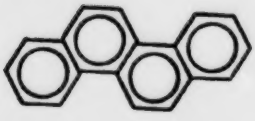


Fluoranthene

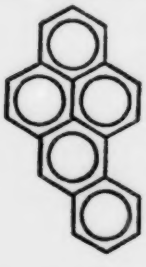
Pyrene



Benzo(a)anthracene



Chrysene



Benzo(a)pyrene

TABLE 3.1.1 BIOLOGICAL PROPERTIES OF THE PAH'S STUDIED

PAH	Molecular Weight	Water Solubility ($mg \cdot L^{-1}$)	Acute[1] Toxicity	Carcino/[1] Mutagencity	Bioaccumulation[2] Potential
Naphthalene	128	31.7	+	-/-	131
Phenanthrene	178	1.29	+	-/-	325
Anthracene	178	0.073	?	-/-	917
Pyrene	202	0.135	?	-/-	--
Fluoranthene	202	0.260	+	-/+	2702
Benzo(a) anthracene	228	0.014	?	+/+	10 109
Chrysene	228	0.002	?	+/+	--
Benzo(a) pyrene	252	0.004	++	+++/+	--

[1]From Sims and Overcash, 1983: + relative degree of effect on organism, - no effect, ? unknown effect.
[2]Ratio of concentration in Daphnia pulex to concentration in water after 24 hours (Southworth et al, 1978).

TABLE 3.1.2 PHYSICAL AND CHEMICAL PROPERTIES OF THE PAH'S STUDIED

PAH	Molecular Weight	Water Solubility[1] ($mg \cdot L^{-1}$)	Vapour Pressure[1] ($N \cdot m^{-2}$ at 20°C)	Log Octanol/ Water Coefficient[1]	Sorption Coefficient[2]
Naphthalene	128	31.7	6.56	3.37	1300
Phenanthrene	178	1.29	9.07×10^{-2}	4.46	23 000
Anthracene	178	0.073	2.61×10^{-2}	4.45	26 000
Pyrene	202	0.135	8.00×10^{-4}	5.32	84 000
Fluoranthene	202	0.260	9.11×10^{-5}	5.33	100 000
Benzo(a) anthracene	228	0.04	6.67×10^{-7}	5.61	260 000
Chrysene	228	0.002	8.40×10^{-5}	5.61	200 000
Benzo(a) pyrene	252	0.004	6.67×10^{-5}	6.04	690 000

[1]From Sims and Overcash 1983 (1 Atm = 101 325 $N \cdot m^{-2}$).
[2]Normalized for percent organic carbon in the adsorbent.

that 0.5 tonne of benzo(a)pyrene per year, on a worldwide basis, reaches the environment through petroleum refinery waste.

The treatment of refinery wastewater results in the accumulation of PAH's, including benzo(a)pyrene, in sludge. Analysis of refinery sludge at the Wastewater Technology Centre revealed 0.7 to 1.7 $mg \cdot kg^{-1}$ of individual PAH's. In Canada, 17.8% of refinery waste is applied to land while 35.7% is disposed of in landfills (PACE, 1981). The repeated input of this waste to soil may result in PAH accumulation in soil if appreciable degradation does not take place.

General industrial activity has resulted in concentrations of benzo(a)pyrene as high as 15 000 $mg \cdot kg^{-1}$ in sediments (Sims and Overcash, 1983), 200 $mg \cdot kg^{-1}$ in soil near an oil refinery (Shabad et al, 1971), 3.5 $\mu g \cdot kg^{-1}$ in polluted river water (Sims and Overcash, 1983) and anthracene concentrations as high as 25 $mg \cdot kg^{-1}$ in plant materials (Edwards, 1983). The natural occurrence of low concentrations of PAH's suggests that mechanisms may have evolved to degrade or otherwise assimilate them into the environment, however the assimilative capacity of the environment for large industrial inputs of PAH is not known.

3.3 Environmental Concerns

3.3.1 Bioaccumulation

Bioaccumalation studies with fish and shellfish have shown that these organisms readily take up PAH's from an aqueous environment. Moreover, Lee et al (1978) found that depuration (elimination from the organism),particularly of high molecular weight compounds, was slow. Southworth et al (1978) found that the degree of bioaccumulation in Daphnia pulex increased by a factor of 10 with each additional ring in the molecular structure. For example, the concentration of naphthalene was roughly 100 times greater in Daphnia tissue than in the surrounding water while that of benzo(a)anthracene was 10 000 times greater. Additional studies by Brown and Weiss (1982) and Neff (1979), indicated that a substantial proportion of PAH's taken up by aquatic organisms was metabolized or depurated when the organism was placed in clean water. However, depuration rates for high molecular weight compounds were sufficiently slow (30 to 58 days for benzo(a)pyrene) to suggest that contaminated organisms might be consumed by predators before depuration is complete and biomagnification might occur.

Little bioaccumulation of PAH's in the tissue of birds and mammals has been documented to date although metabolism of PAH's by mammals has been studied. The hepatobiliary and gastrointestinal tracts have been identified as the main routes of elimination. Metabolism of PAH's to cytotoxins has been identified on a cellular level in man (IARC, 1973).

Uptake of PAH's by plants from solution culture and from soil has been reported, and in some instances has resulted in an accumulation of PAH's in the plant in excess of the soil concentration (Edwards, 1983). Bioaccumulation of PAH's through plants to higher trophic levels, however, has not been documented.

3.3.2 Persistence in soil

The persistence and fate of a PAH in soil are the result of sorption, volatilization, and biological and chemical degradation processes which interact according to the physical and chemical properties of the compound.

Because of their hydrophobic, lipophilic nature, PAH's tend to be strongly adsorbed to soil particles, especially soil organic matter (Means et al, 1980). The movement of PAH's into water courses through entrainment of soil and other particulates has been documented (Harrison et al, 1975), however little movement of PAH's in solution through the soil profile is likely.

The high reactivity of PAH's to oxidizers (singlet oxygen, ozone, chlorine, sunlight) should result in rapid chemical degradation particularly in an aqueous environment. Chemical degradation reactions involving PAH's in the natural environment are generally catalyzed by light and are generally referred to as photochemical degradation. The affinity of PAH's for particulates, however, may reduce their availability for these reactions (Radding et al, 1976 and Mill et al, 1981). Volatilization may be an important factor affecting the fate of naphthalene in the soil or aqueous environment, but the vapour pressure of most other PAH's is low and volatilization is not likely to be appreciable.

Studies have indicated that there may be substantial microbial degradation of PAH's in soil, however the high molecular weight compounds are recalcitrant (Sims and Overcash, 1983). Low water solubility and affinity for soil organic matter may limit the availability of these PAH's for microbial degradation. Thus, the potential for accumulation of the high molecular weight PAH's in soil and sediment is high.

A more detailed description of the processes affecting the persistence of PAH's in soil follows.

4 PROCESSES AFFECTING PERSISTENCE IN SOIL

4.1 Sorption

Sorption to soil is substantial for all PAH's, as a result of their hydrophobicity, and limits the availability of PAH's for other reactions. It is thought to occur predominantly through non-site specific processes such as Van der Waal's forces (Goring and Hamaker, 1972; Lyman et al, 1982). Studies of PAH sorption to soils and sediments (Karickhoff et al, 1979; Hassett et al, 1980; Means et al,1980) have indicated that sorption equilibrium is reached within 24 hours. The concentration of PAH in solution is a linear function of the total PAH concentration until it reaches 60-70% of the water solubility. As the concentration in water approaches the solubility limit the sorption coefficient (i.e., the proportion of PAH not in solution) increases.

Sorption coefficients have been determined for many PAH's on a variety of matrices. The most important matrix property affecting sorption is its organic matter content and sorption coefficients are often reported as normalized for % organic carbon (K_{OC}). Sorption coefficients are highly correlated with the octanol/water coefficient of a compound. In fact, estimates of the K_{OC}'s can be calculated from octanol/water coefficients reported in the literature. The equation $\log K_{OC} = \log K_{OW} - 0.317$ has been suggested by Hassett et al (1980) and $K_{OC} = \log K_{OW} - 0.21$ by Karickhoff et al (1979) for PAH's. Estimates of the concentrations of PAH's in soil water can also be calculated from the sorption coefficient after adjustment for % organic carbon for that specific soil.

The extent or mechanism of sorption to soil may change with time. The increased binding of certain pesticide residues to soil with time has been well documented and is described by Khan (1980, 1982). The author suggests that bound residues (either parent compound or metabolite) may be chemically associated with soil organic matter through complexation or co-polymerization, or may be physically bound by entrapment within the molecular sieve-like structure of soil humus. Chemical interaction of PAH's with soil organic matter may be stimulated through the formation of PAH free radicals upon absorption of light, or may involve PAH metabolites such as phenolics and hydroquinones. Khan (1982) indicated that bound pesticide residues can be released from soil by microbial activity and/or taken up by plants. No assessment of the nature or bioavailability of PAH's has been made.

4.2 Volatilization

For most PAH's volatilization occurs from the fraction that is not sorbed, i.e., from soil water (Sims and Overcash, 1983). For naphthalene, volatilization from a solid state (sublimation) may also be important.

The rate of volatilization of chemicals from water bodies has been described by simple first order kinetics (Sims and Overcash, 1983). In soil, however, the process is complicated by sorption and diffusivity. As sorption of most PAH's is high, little PAH is available in soil water for volatilization. In addition, the susceptibility of most PAH's to

volatilization from the water phase, as indicated by their Henry's Law constants (vapour pressure/solubility), is low.

4.3 Photochemical Degradation

Photochemical degradation involving singlet oxygen is the most important form of chemical degradation of PAH's in the environment. In water the process is a linear function of light intensity as well as being oxygen and temperature dependent (Radding et al, 1976) and exhibits first order kinetics (Mill, 1980). Little is known about the photoreactivity of sorbed hydrophobic chemicals, however evidence exists that sorption both enhances (Radding et al, 1976; Hansch, 1980; Zepp, 1980) and inhibits (Radding et al, 1976; Hansch, 1980; Sims and Overcash, 1983) the photochemical degradation of PAH's.

4.4 Microbial Degradation

Microbial degradation of an easily metabolized substrate typically results in an exponential decrease in concentration with no discernable lag phase. A lag phase, or period of microbial adaptation, may occur if a substrate is not easily degraded. Microbial adaptation refers to the process by which a microbial population adapts to new growth conditions, for example through the synthesis of specific enzymes or elaboration of complete pathways required to degrade a substrate (Lynch and Poole, 1979). Although some factors may limit further growth, the population will retain the metabolic potential for continued growth on that substrate. Upon further addition of the substrate, degradation by an adapted population will proceed with a shorter lag period and a higher rate constant than with the initial substrate addition. Repeated addition of a substrate can thus be used to test if microbial adaptation to a substrate is significant. The presence of easily metabolized PAH's may stimulate the degradation of more recalcitrant PAH's through enhancing microbial adaptation.

As a result of microbial adaptation the population of microorganisms capable of degrading the substrate increases exponentially, accelerating the rate of degradation, and resulting in a decrease in the substrate concentration. An exponential decline in the rate of degradation occurs as the substrate concentration, or some other factor, becomes limiting. Degradation can be limited by a number of factors including the availability of the added substrate with time and the concentration of other substrates or nutrients. It has been observed that microbial degradation is kinetically limited with reduced metabolic activity (as a result of insufficient energy transfer or enzyme stimulation) at substrate concentrations of 0.05 to 0.10 $mg \cdot kg^{-1}$ (Kretscheks and Krupka, 1984). Microbial degradation in soil may be further limited by the concentration of substrate in soil water, and therefore available to microorganisms, rather than by the total concentration in the soil.

Degradation of complex or recalcitrant molecules is frequently linear over an extended period of time. This type of response occurs when degradation is not enhanced by the adaptation of a specific enzyme system or pathway and microbial population dynamics are not related to the

substrate concentration. Degradation of these compounds occurs randomly by the microbial population as a whole and may be enhanced by the presence of other substrates through cooxidation or through a general increase in the microbial population. This type of response may also occur when substrate concentrations available for microbial degradation are very low and fail to stimulate specific enzyme systems.

As the rate of degradation of most compounds is dependent on concentration, it is influenced by all other factors affecting the concentration of those compounds in soil.

5 MICROBIAL DEGRADATION STUDIES WITH PAH'S

PAH's are degradable, to varying degrees, by soil microorganisms. Sims and Overcash (1983), Radding et al (1976) and Neff (1979) reviewed microbial degradation studies in which diverse experimental approaches and techniques were used. Studies ranged in complexity from isolated microbial cultures grown on specialized media to the incubation of environmental samples. Studies of isolated microbial cultures allow the researcher to identify microbial strains responsible for degradation, determine mechanisms involved and isolate metabolites. Studies in complex environments include more variables, i.e. more of the components of "fate", and therefore are closer to real world situations. However, the results of complex systems are frequently difficult to interpret. A brief review of microbial degradation studies follows.

5.1 Pure Culture Studies

Mechanisms of degradation have been defined for certain PAH's with soil microorganisms grown on specialized media. Unsubstituted PAH's are typically degraded through the incorporation of molecular oxygen into the aromatic ring structure by oxidizing enzymes. Monooxygenases are enzymes that catalyze the incorporation of a single oxygen atom into the ring, and result in the formation of a cytotoxic arene oxide (epoxide) intermediate. Dioxygenases catalyze the incorporation of both atoms of molecular oxygen into the ring to form a biologically inert dioxetane intermediate. Both intermediates undergo further dihydroxylation steps leading to enzymatic cleavage of the aromatic ring and degradation of the resulting phenols and carboxylic acids (Sims and Overcash, 1983).

Metabolism of PAH's in mammalian and fungal cells typically proceeds by the monooxygenase pathway while metabolism by bacteria proceeds by the dioxygenase pathway. Jeffery et al (1975) identified the degradation of naphthalene by a soil bacterium (Pseudomonus sp.) through dioxygenase metabolism and Gibson et al (1975) induced a mutant strain of soil bacterium (Beijerinckia sp.) to oxidize benzo(a)pyrene and benzo(a)anthracene by a dioxygenase enzyme system. With the soil fungi Cunninghamella elegans, however, metabolism of benzo(a)pyrene (Cerniglia and Gibson, 1979) and of anthracene and phenanthrene (Cerniglia and Yang, 1984) was found to occur through a monooxygenase enzyme system. The dihydrodiol metabolites produced by the soil fungi differed in absolute stereochemistry from the cytotoxic metabolites produced in mammalian systems and may differ significantly in their biological activity.

The biological activity of PAH metabolites produced by soil microorganisms has not been positively identified. Evidence suggests, that the soil microbial community may metabolize PAH's through both the mono- and dioxygenase metabolic pathways producing cytotoxic and inactive metabolites. As fungi predominate in soils of low pH, liming a soil may encourage the activity of soil bacteria and thus favor the dioxygenase metabolic pathway.

Culture studies such as those described above provide no information on the occurrence or persistence of PAH metabolites in actual soil. Herbes and Schwall (1978) suggested that, in sediment, initial

oxygenation steps are rate determining and that the intermediates are further oxidized as soon as they are produced. Few studies have attempted to identify metabolites in soil.

Culture studies have provided other insights that may be applied to soil treatment of PAH's. The activity of enzymes responsible for the degradation of PAH's may be induced through application of metabolite analogues. For example, the degradation of naphthalene was stimulated through induction of the required enzyme system by salicylate, itself a product of naphthalene metabolism (Sims and Overcash, 1983). Pre-exposure or addition to the soil of PAH metabolites may play an important role in microbial adaptation to PAH's.

Through the incubation of mixtures of organic molecules it has been observed that 4 and 5 ring PAH's, which do not themselves support microbial growth, can be degraded in the presence of other PAH's which do support growth and act as co-substrates. This cooxidation of PAH's may be an important factor for PAH degradation in a mixed medium such as sludge and soil.

5.2 Rates of PAH Degradation in Soil

Studies involving environmental samples such as soil, sediment and sludge provide supporting evidence for microbial degradation of PAH's. Unfortunately, the matrix, environmental conditions, experimental and analytical techniques have varied so that rates of degradation are currently poorly defined. Sims and Overcash (1983) reported half lives that ranged from 0.12 to 125 days for naphthalene, 3.3 to 175 days for anthracene, 2.5 to 26 days for phenanthrene, 3 to 35 days for pyrene, 44 to 182 days for fluoranthene and 4 to 6250 for benzo(a)anthracene. Studies with chrysene and benzo(a)pyrene have reported half lives as low as 5.5 and 2 days, respectively, but other reports have indicated that no degradation took place.

Most studies report first order, or concentration dependent, kinetics. Gardner et al (1979) studied PAH degradation in marsh sediment and reported half lives in the upper range reported by Sims and Overcash (1983) for anthracene and fluoranthene (108 to 175 and 105 to 182 days, respectively). The half lives for benzo(a)anthracene and benzo(a)pyrene were 118 to 252 and 30 to 420 days, respectively. Herbes (1981) reported half lives in the lower range reported by Sims and Overcash (1983) for naphthalene, anthracene and benzo(a)anthracene in pre-exposed sediment collected downstream from a coking plant discharge (approximately 0.4, 1.8 and 8.8 days, respectively), however in this study, no degradation of benzo(a)pyrene was observed.

Factors that have been observed to affect the degradation of PAH's are temperature, pH, aeration, moisture, PAH concentration and previous exposure (Sims and Overcash, 1983). Degradation tends to increase, as does overall microbial activity, under conditions of ideal temperature, pH and moisture. It has not been observed under anaerobic conditions. The rate of degradation is generally greater at higher concentrations and is enhanced by previous exposure to the chemical.

5.3 Fate Studies

Some studies in freshwater and marine environments have attempted to elucidate the fate of PAH's through the use of ^{14}C labelled isotopes.

Lee et al (1978) studied the significance of removal processes in a marine environment. Volatilization and microbial degradation were the primary methods of removal for naphthalene, anthracene and phenanthrene, while sedimentation and photochemical oxidation accounted for most of the losses of chrysene, benzo(a)anthracene and benzo(a)pyrene. The authors did not study microbial degradation in sediments but suggested that the high molecular weight PAH's may persist in sediment for a long time.

Herbes and Schwall (1978) and Herbes (1981) studied PAH degradation in freshwater sediments. They found that binding of ^{14}C in microbial cells was a significant component of the fate of naphthalene and anthracene. Herbes and Schwall (1978) found approximately 7% of added naphthalene remained unchanged in sediment after 24 hours, 1% of the ^{14}C remained as water soluble metabolites, 30% was bound in microbial cells and 50% was degraded to CO_2. After 24 hours approximately 78% of added anthracene remained in sediment; 2% of the ^{14}C remained as water soluble metabolites, 5% was bound and 2% was degraded to CO_2. After 7 days, however, 29% of the ^{14}C remained as anthracene and 23% was bound in microbial cells. Water soluble and CO_2 fractions accounted for about 6 and 4%, respectively. Total recovery of ^{14}C from the system was variable and after 7 days was less than after 24 hrs. Benzo(a)anthracene and benzo(a)pyrene were recalcitrant even in preexposed sediments. Approximately 77% of added benzo(a)anthracene remained after 14 days, 1-2% was transformed to each of the water soluble, bound and CO_2 fractions. After 26 days approximately 27% of the ^{14}C remained as benzo(a)anthracene and 16% was degraded to CO_2. There was no change in the water soluble and bound fractions. Total recovery was reduced after 26 days and the authors suggested that the sample may have become anaerobic during the extended incubation resulting in loss as $^{14}CH_4$. The primary mechanism for removal of benzo(a)anthracene appeared to be microbial degradation. Little degradation of benzo(a)pyrene occurred in 26 days with about 75% of ^{14}C remaining as the parent PAH, about 4% as water soluble metabolites, 0.4% bound and 0.2% evolved as CO_2. Although it was noted that the increase in water soluble metabolites was not statistically significant, it is interesting to note that it accounted for the largest proportion of transformation products.

5.4 Limitations of Microbial Degradation Studies

In general, incubation studies have shown that microbial degradation is an important process for low molecular weight PAH's and may also be important for higher molecular weight PAH's. There is currently a need, however, for statistically reliable degradation data, for data relating biodegradation in soil to other fate mechanisms, for the determination of metabolites and their fate, for the development of techniques to optimize the degradation of high molecular weight PAH's and for field verification of degradation results. So far techniques have not been standardized and degradation rates in soil are poorly defined.

Incubation studies have a number of inherent limitations. Added chemicals may be more available to microorganisms than indigenous chemicals and degradation may be over-estimated or toxicity may occur. Shifts in the microbial community, especially with long incubation periods, may not be representative of those occurring in the natural environment.

There are some advantages to performing incubation studies, however. The use of pure chemical additions to soil increases analytical precision, and provides information on degradation that is not dependent on the properties of a particular waste matrix. The use of controlled environmental conditions makes it possible to repeat studies under similar conditions. Parameters can then be varied individually and results compared to a common reference point. Thus the processes involved in degradation may be more easily interpreted. Although these conditions are not identical to natural conditions they provide important basic information which can then be verified through field experimentation.

6 OBJECTIVES

Appreciable amounts of PAH's enter the soil environment from industrial sources, including the combustion of coal and oil to generate heat and power, coke production and in the oil refining process. The disposal of oily wastes may result in the addition of PAH's to soil. Because of the toxic, mutagenic and/or carcinogenic properties of PAH's their fate in the environment is of concern.

PAH degradation studies indicate that PAH's are degraded in soil but high molecular weight PAH's, for example benzo(a)anthracene and benzo(a)pyrene, may degrade very slowly. Other processes, such as volatilization, leaching, photochemical degradation and irreversible sorption, may also affect the persistence of PAH's added to soil. Studies with pure cultures indicate that some degradation products of PAH's in soil may be environmentally hazardous. The persistence of PAH's in soil and the significance of the various fate mechanisms must be understood to develop appropriate technology for disposal of these wastes to land.

The objectives of the current study were to determine the kinetics of disappearance of a mixture of PAH's from unacclimated soil and to assess biodegradation, chemical degradation, volatilization and sorption as fate mechanisms. Detailed identification of metabolites and determination of their toxicities was beyond the scope of this study.

The study was designed in two parts as follows:

Part I

A mixture of 8 PAH's (naphthalene, phenanthrene, anthracene, pyrene, fluoranthene, benzo(a)anthracene, benzo(a)pyrene and chrysene) was incubated at two concentrations in previously uncontaminated soil to determine the kinetics of PAH disappearance from soil and estimate order of reaction and rate constants with statistically defined confidence limits.

Part II

Anthracene and benzo(a)pyrene, labelled with ^{14}C, were incubated in soil at two levels of application to determine the proportions affected by biodegradation, chemical degradation, volatilization and adsorption.

7 METHODOLOGY

The eight PAH's, naphthalene, phenathrene, anthracene, pyrene, fluoranthene, benzo(a)anthracene, chrysene and benzo(a)pyrene, were selected for the fingerprint study (Part I) based on their occurrence in environmental samples and their range of physical and chemical properties. From these eight, anthracene and benzo(a)pyrene were selected for the ^{14}C study (Part II) because information in the literature indicated that they would be representative of a non-persistent and persistent PAH, respectively. In addition, benzo(a)pyrene is of particular interest in environmental studies because it is a potent carcinogen.

A bulk sample of surface (Ah) horizon Donnybrook sandy loam was taken from a pasture area in an agricultural community approximately 100 km northwest of Toronto, Ontario. The moist soil (17.1% dry weight) was put through a No. 10 mesh (2 mm) seive and stored in polyethylene bags. Soil characteristics were as follows: 63% sand, 31% silt, 6% clay, 0.067 mol $(p^+) \cdot kg^{-1}$ cation exchange capacity, 2.3% organic carbon, pH 7.0, 1305 $mg \cdot kg^{-1}$ total Kjeldal nitrogen, 465 $mg \cdot kg^{-1}$ total phosphorus, 20 $mg \cdot kg^{-1}$ exchangeable K.

Naphthalene (99%) was obtained from the J.T. Baker Chemical Co. Fluoranthene and phenanthrene (fluorescent grade) were obtained from the Eastman Kodak Inc. and benzo(a)anthracene (99%), benzo(a)pyrene (98%), pyrene (99%), chrysene (98%) and anthracene (99%) were obtained from Aldrich Chemical Co. Benzo(a)pyrene (7,10-^{14}C) and anthracene (9-^{14}C) of 98% radiochemical purity were obtained from California Bionuclear Corp. Stock solutions were made of the PAH's in toluene and isopropyl alcohol.

In order to mix the PAH's evenly throughout the experimental soil, PAH stock solutions were added to small amounts of soil at two concentrations. The solvents were allowed to evaporate and the treated soil was then mixed into fresh soil at a rate of 8% (dry weight basis) to bring the final concentration of each PAH in soil to 5 and 50 $mg \cdot kg^{-1}$. The mixtures were then brought to 29.2% moisture which was 60% of the soil water holding capacity (WHC).

A description of the methodology specific for each part of the study follows.

7.1 Part I

7.1.1 Experimental

In the first part of the study a mixture of the eight PAH's was added to the soil at concentrations of 5 and 50 $mg \cdot kg^{-1}$ for each PAH (total PAH loading of 40 and 400 $mg \cdot kg^{-1}$).

A total of 2700 g of soil was prepared for each treatment and divided into three replicates of 900 g each. Each replicate was further subdivided among three 500 mL erlenmyer flasks (300 g/flask). Samples taken from the three flasks representing one replicate were bulked for analysis. A total of 27 flasks were prepared, stoppered with absorbent cotton, and placed in a controlled temperature chamber held at 20°C for 400 days. The moisture content of the soil was maintained by monitoring total

soil and flask weight and adding water as required (approximately bi-weekly). Measurements of samples taken for analysis indicated that the moisture content was maintained at 60% water holding capacity ±5%. Light was not a controlled parameter in this study, however, the samples were unintentionally illuminated with 118 lux. (11 foot candles) continuously for approximately 40 days during the study.

Initial sampling frequency was roughly logarithmic, to adequately describe the disappearance of PAH's which are rapidly removed from soil. Later, sampling was performed biweekly and monthly as judged necessary from preceding analyses. Sampling was performed for a total of 25 periods. For each period the soil was mixed thoroughly and 5 g samples (wet weight) were removed from each flask. The 15 g (wet weight) sample from each replicate was placed in a glass vial and frozen pending analysis.

7.1.2 Analytical

The soil samples were analysed for the 8 PAH's using the method described in Appendix A. It was a modification of a method developed by Afghan and Wilkinson (unpublished report, 1982) for the analysis of PAH's in sediments. In summary, it involved extraction of 8 g of soil with acetone using a Polytron homogenizer. The resulting extract was then partitioned between water and hexane. The hexane supernatant was concentrated and cleaned up by gel permeation chromatography, which separates molecules according to their size. The PAH-containing fraction was then analyzed by reverse phase high performance liquid chromatography (HPLC) using a combination of fluorescence and U.V. detectors.

With the exception of naphthalene, which evaporated from the soil very quickly, recoveries of known PAH additions to soil ranged from 77 to 99%. Relative standard deviations for the recoveries ranged from 4.2 to 6.8% at a spiking level of 10 $\mu g \cdot g^{-1}$. The detection limit depended on the compound, but the average was about 0.01 $\mu g \cdot g^{-1}$.

7.1.3 Statistical

Statistical analysis of the data was performed by fitting a kinetic model to the data by non-linear regression analysis. Microbial degradation has often been described by zero and first order kinetics for recalcitrant and easily degraded substrates, respectively. In this study a general power rate model was used:

$$\frac{-dC}{dt} = KC^n$$

where K is a rate constant C is concentration and n is the order of the reaction (Goring and Hamaker, 1972; Lyman et al, 1982). The model was fitted to the data by iterative non-linear regression involving Gauss-Marquardt estimation procedures and using a computer subroutine (UWHAUS) developed at the University of Wisconsin by Meeter and Wolfe (1965). Initial parameter estimates were supplied to the subroutine. The differential form of the model was then integrated numerically to predict concentrations. Predicted and observed concentrations were compared by

regression analysis and the residual mean squares were calculated. Parameter estimates were then revised iteratively to improve the fit until the relative change in the sum of squares was less than 1×10^{-4}.

Use of the power rate model assumes that a decrease in concentration occurs throughout the incubation period. Microbial degradation, however, may occur after a period of adaptation in which little degradation takes place but growth occurs for that portion of the microbial population which can degrade the substrate. Graphs of the data were assessed visually for evidence of an initial lag period and regression analysis was then performed only on subsequent data.

A further limitation of this model was that in some cases the rate constants and reaction order were highly correlated and estimates of the rate constants were imprecise. To obviate this problem the model was subsequently fitted to the data using fixed reaction orders as well as the order of reaction estimated by regression. Residual mean squares were then compared to confirm best fit. The data were plotted on a logarithmic scale to assist in visual assessment of the reaction order.

7.2 Part II

7.2.1 Experimental

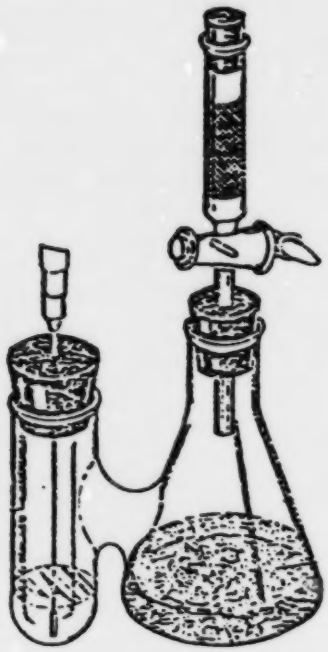
In the second part of the study ^{14}C labelled benzo(a)pyrene and anthracene were added to soil at rates of 5 and 50 $mg \cdot kg^{-1}$. The additions were made to soil with and without the addition of 5000 $mg \cdot kg^{-1}$ $HgCl_2$ to compare results for sterilized and biologically active soil. Each treatment was replicated three times.

Benzo(a)pyrene or anthracene was added to the soil as described for Part I but with the further addition of 1.38×10^7 dpm (disintegrations per minute) of 7, 10-^{14}C benzo(a)pyrene or 1.2×10^7 dpm of 9-^{14}C anthracene in 1 mL toluene. All soils were brought to 29.2% moisture (dry weight) and placed in Bellco biometer flasks, illustrated in Figure 7.2.1 (Bartha and Pramer, 1965). The biometer flasks consisted of a modified 250 mL Erlenmeyer flask with a 50 mL reservoir attached via a connecting arm. The reservoir contained 10 mL of 0.2 N NaOH (CO_2-free) for trapping CO_2 evolved from the sample in the main flask. The reservoir was sealed with a stopper fitted with a 15 gauge needle for adding and removing alkali without disturbing the sample. The main flask was sealed with a stopper fitted with an ascarite filled tube which was opened via a stopcock to permit entry of CO_2-free air into the flask. A plug (approximately 0.9 g) of type 1338 polyurethane was placed into the connecting arm of the flask to absorb volatilized PAH. The flasks were stored in a Precision Scientific incubator at 20°C and aerated twice weekly by injecting 200 mL of air through the ascarite filter. Triplicate blank flasks were prepared exactly as the treatment flasks but without soil samples.

The benzo(a)pyrene study included three replicates of each treatment to be incubated for 145 days, as well as 3 flasks containing the 5 $\mu g \cdot g^{-1}$ treatment and 3 containing the 50 $\mu g \cdot g^{-1}$ treatment which were to be "sacrificed" at intervals during the incubation period. NaOH was

sampled for analysis ^{14}C and replaced bi-weekly. In the case of anthracene, three replicates were incubated for 71 days, three flasks per treatment were sacrificed during the incubation period and NaOH was sampled and replaced weekly.

FIGURE 2. FLASK FOR MONITORING THE PRODUCTION BY SOIL OF CARBON DIOXIDE (Bartha and Pramer, 1965)



7.2.2 Analytical

Details of the ^{14}C analytical procedures are given in Appendix B. The procedures are summarized below.

Analysis of NaOH for ^{14}C as CO_2 was performed by removing a 1 mL sample from the biometer flask reservoir and extracting it with hexane to remove any non-polar ^{14}C material. The hexane (non-polar) and NaOH fractions were mixed separately with Phase Combining System (PCS) scintillation cocktail from Amersham Corp. and ^{14}C was measured with a LKB model 1217 liquid scintillation counter.

The soil samples were removed from the flasks and extracted as described for the first part of the study using a Polytron homogenizer. In this part of the study, however, the fraction partitioned into water, as well as that in hexane, was reserved for analysis. The polyurethane plug was extracted with hexane using a Soxhlet apparatus. All extracts were analyzed for ^{14}C by liquid scintillation counting.

Identification of ^{14}C occurring as benzo(a)pyrene or anthracene in extracts was performed by thin layer chromatography (TLC). The extract

and a benzo(a)pyrene or anthracene standard were applied to each of two Whatman LK5DF silica gel plates which were developed separately to a distance of 12 to 14 cm in 1:1:1 hexane/acetone/toluene(retention factor - R_f 0.9) or hexane (R_f 0.3). To determine whether any radioactivity due to metabolites could be detected at different locations on the plate, 1 cm sections of the TLC strip were recovered individually, eluted with toluene and counted. Following extraction, residual ^{14}C in the soil and plug were analyzed by complete combustion with a Harvey biological material oxidiser, trapping and counting as CO_2.

7.2.3 Statistical

The distribution of ^{14}C between evolved CO_2, extracts and residual components of the system at the end of the incubation period was assessed by construction of a mass balance table. Data from the treatments with and without $HgCl_2$ at the low and high rates of PAH addition were compared for each component of the system by two way analysis of variance and the Duncan's multiple range test for treatment means.

The model that was identified as appropriate for estimating the loss of a PAH benzo(a)pyrene or anthracene from soil in Part I was fitted to the data of extracted PAH in Part II to compare the behaviour of the systems in Parts I and II.

8 RESULTS AND DISCUSSION

8.1 Part I - The Persistence of PAH Compounds

Figures 8.1.1 to 8.1.8 illustrate the concentrations of the PAH's in soil over the 400-day period for the two rates of PAH addition and control soil. The concentration of PAH's generally decreased with time, however in some cases the change in concentration was very small over the 400-day period.

The data has been plotted against a logarithmic scale for concentration to facilitate comparison of the responses at two levels of PAH addition. The slope in these plots is indicative of the first order rate constant. Plots of similar slopes for the 5 and 50 $mg \cdot kg^{-1}$ treatments indicate that the rate constant is independent of the initial concentration (i.e., the amount added). A plot that is approximately linear against a logarithmic axis indicates that the response was proportional to the concentration (i.e., concentration to the power one) of PAH remaining in soil throughout the incubation period (first order kinetics).

Deviation of the plots from a straight line may occur because the response is not proportional to concentration. Alternatively the response may reflect a change in kinetics with time.

Inspection of the figures indicated that the low molecular weight PAH's, naphthalene and phenanthrene, did not appear to require a lag period (i.e., the decrease in concentration began without delay). For pyrene, fluoranthene and the 50 $mg \cdot kg^{-1}$ treatment of anthracene, lag periods were observed and the rate of decline in concentration was slower than for the low molecular weight PAH's. In some cases it appeared that, in the early stages of incubation, the decreases in concentration were first order with the same rate constant for both the 5 and 50 $mg \cdot kg^{-1}$ treatments. The normalized plots (plots of concentration divided by initial concentration) in Figures 8.1.2a, 8.1.4a and 8.1.5a illustrate that, for several PAH's, first order rate constants were independent of initial concentration in the early stages of incubation but were not consistent over the entire incubation period.

The most persistent PAH's, benzo(a)anthracene, chrysene and benzo(a)pyrene, tended to be those with the highest molecular weight, and sorptivity and the lowest solubility. These physical properties may have limited microbial degradation of the PAH's. The first order rate constant for chrysene was independent of initial concentration (Figure 8.1.7a). For benzo(a)anthracene and benzo(a)pyrene, however, the rate constant was greater with the 50 $mg \cdot kg^{-1}$ treatment than with the 5 $mg \cdot kg^{-1}$ treatment.

Figures 8.1.1 to 8.1.8 illustrate the variability encountered in the data, which averaged approximately 20% and was typical for a heterogeneous matrix such as soil. This variability affected the precision of parameter estimation and, particularly where decreases in the concentration occurred slowly, confidence intervals for the parameters were wide.

Finally, the figures illustrate the background concentration of each PAH in soil. The exception is naphthalene for which no background concentration was detected. For most PAH's the concentration decreased from 5 $mg \cdot kg^{-1}$ to background (control) levels within the 400-day incubation period, the exceptions being benzo(a)anthracene, chrysene and benzo(a)pyrene. The concentration was reduced from 50 $mg \cdot kg^{-1}$, to background levels, however, only for naphthalene, and possibly phenanthrene within 400 days.

A summary of the estimated kinetic parameters and their 95% confidence intervals is provided in Table 8.1.2. The adaptation phase indicated in Table 8.1.2 was determined by visual assessment of the figures while reaction orders and rate constants were estimated by regression analysis. Confidence intervals for each parameter are indicated in brackets and parameter values are significantly different in cases where the confidence intervals do not overlap. For cases in which change in concentration was minimal (each of benzo(a)anthracene, chrysene and benzo(a)pyrene with the 5 $mg \cdot kg^{-1}$ treatment) it was impossible to determine precise reaction orders. Statistical analysis generally supported the visual interpretation of the graphs.

To facilitate comparison of the results with those found in the literature, first and zero order models were also fitted to the data and half lives were calculated. These predictions and the range of half lives reported by Sims and Overcash (1983) are presented in Table 8.1.3. In general, the results of this study agree with observations in the literature. A detailed discussion of the kinetic information presented in these tables follows.

8.1.1 Naphthalene

Because of its volatility, the analytical results for naphthalene were extremely variable (Figure 8.1.1) and the data was not suitable for kinetic modelling. A large proportion of naphthalene apparently was lost through volatilization during spiking and sample preparation, as reported in Appendix A. The data did indicate that naphthalene was lost quickly from soil, with no naphthalene detected after 12 days. The plot of normalized data in Figure 8.1.1a suggests that first order rate constants were independent of initial concentration but data from the 5 mg•kg level of addition was variable.

TABLE 8.1.2 KINETICS PARAMETERS ESTIMATED FOR THE DISAPPEARANCE OF PAH'S FROM SOIL WITH TWO RATES OF PAH ADDITION

PAH	Adaptation Phase (days)	Reaction Order*	Rate* Constant (day^{-1})
Phenanthrene (0 to 19 days)			
5 mg kg^{-1}	0	0.33 (-0.06 to 0.72)	0.1419 (0.1193 to 0.1645)
50 mg kg^{-1}	0	0.00 (-0.61 to 0.61)	1.508 (1.295 to 1.720)
(26 to 400 days)			
5 mg kg^{-1}	NA	0.00 (-4.55 to 4.55)	0.0007 (0.0004 to 0.0009)
50 mg kg^{-1}	NA	0.93 (0.08 to 1.79)	0.0061 (0.0050 to 0.0072)
Anthracene (0 to 400 days)			
5 mg kg^{-1}	0	1.42 (1.15 to 1.69)	0.0268 (0.0233 to 0.0303)
50 mg kg^{-1}	19	1.31 (1.14 to 1.48)	0.0097 (0.0088 to 0.0106)
Fluoranthene (0 to 400 days)			
5 mg kg^{-1}	19	1.40 (1.05 to 1.76)	0.0223 (0.0183 to 0.0262)
50 mg kg^{-1}	19	1.37 (1.08 to 1.67)	0.0131 (0.0111 to 0.0152)
Pyrene (0 to 400 days)			
5 mg kg^{-1}	26	1.04 (0.65 to 1.43)	0.0190 (0.0156 to 0.0224)
50 mg kg^{-1}	26	1.08 (0.70 to 1.46)	0.0220 (0.0177 to 0.0262)
Benzo(a)anthracene (0 to 400 days)			
5 mg kg^{-1}	40	2.72 (1.29 to 4.15)	0.0006 (0.0005 to 0.0007)
50 mg kg^{-1}	40	1.00 (0.55 to 1.45)	0.0080 (0.0070 to 0.0089)
Chrysene (0 to 400 days)			
5 mg kg^{-1}	0	0.00 (-2.98 to 2.98)	0.0081 (0.0069 to 0.0092)
50 mg kg^{-1}	0	0.00 (-0.68 to 0.68)	0.1179 (0.1065 to 0.1293)
Benzo(a)pyrene (0 to 400 days)			
5 mg kg^{-1}	0	1.01 (-5.64 to 7.66)	0.0009 (0.0005 to 0.0012)
50 mg kg^{-1}	0	0.00 (-0.95 to 0.95)	0.1031 (0.0924 to 0.1139)

* Figures in brackets are 95% confidence intervals calculated for the kinetic parameters.

TABLE 8.1.3. A COMPARISON HALF LIVES CALCULATED ON THE BASIS OF FIRST OR ZERO ORDER MODELS FROM DATA COLLECTED IN THIS STUDY WITH THOSE REVIEWED BY SIMS AND OVERCASH (1983)

PAH	Half Life 5 $mg \cdot kg^{-1}$ added (days)	Half Life 50 $mg \cdot kg^{-1}$ added (days)	Range of Half Lives from Sims and Overcash (days)
Naphthalene	12[a]	12[a]	0.12 to 125
First Order			
Phenanthrene	9.7	14	2.5 to 26
Anthracene	17	45	3.3 to 175
Fluoranthene	39	34	44 to 182
Pyrene	58	48	3 to 35
Benzo(a)anthracene	240	130	4 to 6250
Zero Order			
Chrysene	328	224	5.5 +
Benzo(a)pyrene	347	218	2 +

[a]Total residence time in soil.

There is much evidence in the literature to suggest that naphthalene loss from soil is due to microbial degradation. For example, soil micoorganisms have been isolated that degrade naphthalene (Fernley and Evans, 1958; Gibson, 1968). Herbes and Schwall (9178) estimated approximately 50% of ^{14}C-naphthalene loss from sediment was due to microbial degradation, measured as $^{14}C\text{-}CO_2$ trapped in alkali. However, the high vapour pressure and low sorption coefficient of naphthalene suggests that volatilization may also have been significant.

Preliminary studies were performed with ^{14}C-naphthalene and indicated that volatilized naphthalene, as well as CO_2, was trapped by alkali in incubation experiments. Herbes and Schwall (1978) did not report removal of naphthalene from their alkali solution, or otherwise account for volatilized naphthalene, and thus may have overestimated microbial degradation.

FIGURE 8.1.1 NAPHTHALENE DISAPPEARANCE FROM SOIL

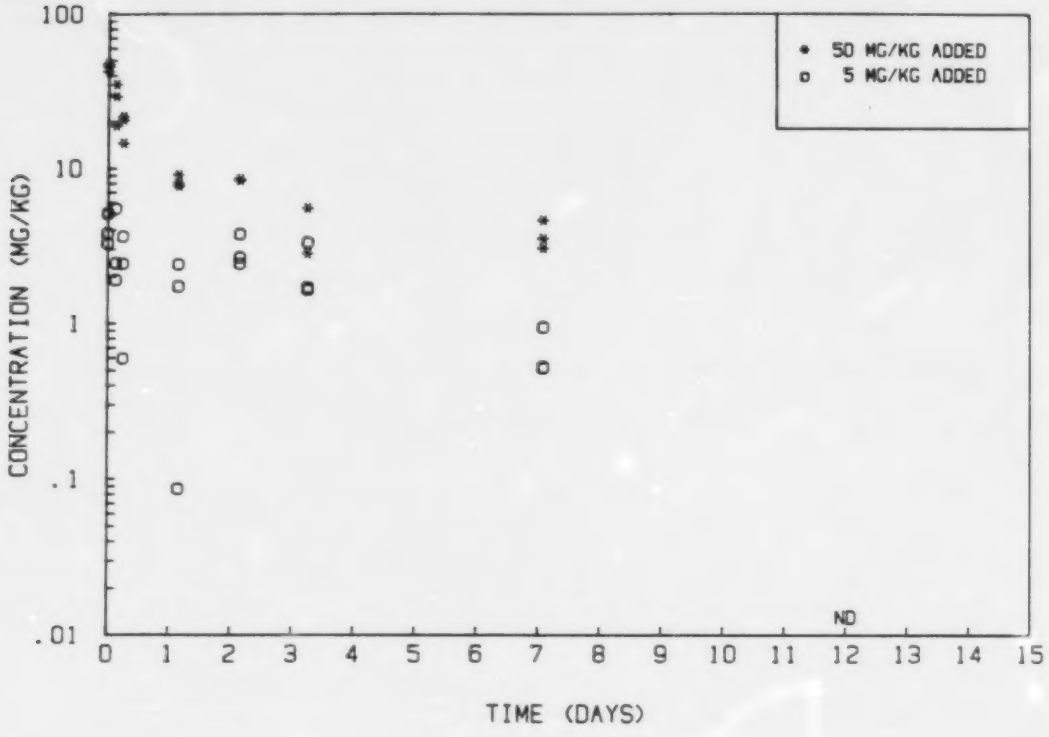
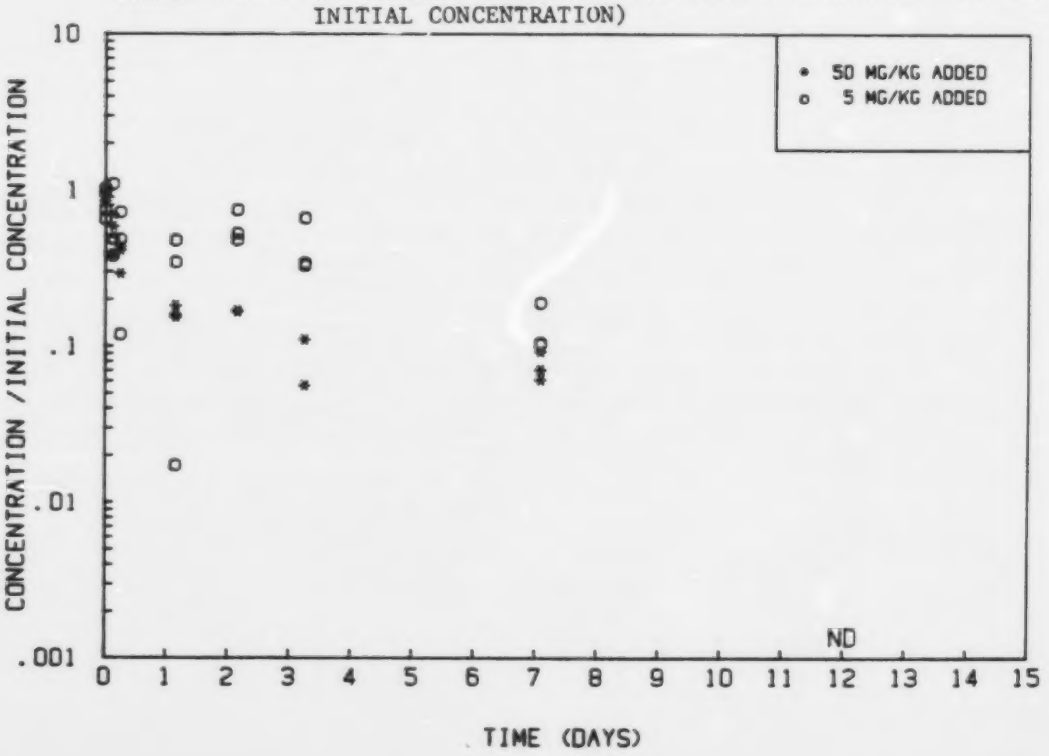


FIGURE 8.1.1a NAPHTHALENE DISAPPEARANCE FROM SOIL (CONCENTRATION/ INITIAL CONCENTRATION)



The short residence time observed in this study is consistent with other studies, as indicated in Table 8.1.3. Based on the results of this study, one would not expect naphthalene to accumulate in soil.

8.1.2 Phenanthrene

The concentration of phenanthrene in soil appeared to decrease rapidly within the first 26 days of incubation but slowly for the rest of the incubation period. Within the first 26 days approximately 96% of added phenanthrene had disappeared, as illustrated in Figure 8.1.2 A reasonable fit of this model could not be obtained using data for the full 400 day period, therefore modelling was performed in two stages. Data from the first 26 days were best described by a 0.33 order model with a rate constant of 0.0415 day^{-1} for the 5 $mg \cdot kg^{-1}$ treatment and with a zero order model and rate constant of 1.508 day^{-1} for the 50 $mg \cdot kg^{-1}$ treatment (Table 8.1.1). Thus, the initial disappearance of phenanthrene appeared to occur at a near linear rate. If the removal mechanism was microbiological in nature, the linear rate would indicate that the degrading population was not experiencing growth on the substrate, and that the concentration was not rate limiting. The plot of normalized data in Figure 8.1.2a indicates that if first order kinetics are assumed,the initial first order rate constant was independent of the initial concentration.

After approximately 26 days the rate of disappearance of phenanthrene appeared to change and the rate constant was no longer independent of the amount of phenanthrene added. Data from the period 26 to 400 days were described by a zero order model with a rate constant of 0.0007 day^{-1} for the 5 $mg \cdot kg^{-1}$ treatment but a 0.93 order model with a rate constant of 0.0061 day^{-1} for the 50 $mg \cdot kg^{-1}$ treatment. The concentration of phenanthrene remaining from the 5 $mg \cdot kg^{-1}$ treatment approached the background concentration in soil and little change in concentration occurred after 26 days. The low concentration may have limited microbial degradation processes. With the 50 $mg \cdot kg^{-1}$ treatment the concentration continued to decrease after 26 days, however at a lower rate than initially observed and with an apparent change in order of reaction. This effect was not simply related to the concentration remaining in soil at that time, i.e., was not the same as the response at a similar concentration with the 5 $mg \cdot kg^{-1}$ treatment, but may have been related to some change in the sorption of phenanthrene on soil surfaces or to changes within the microbial population. As a drop in degradation rate did not occur after 26 days with other PAH's, it was unlikely that it was due to an overall reduction in microbial activity.

Phenanthrene has a low Henry's Law constant (vapour pressure/ solubility) therefore, relatively little of the decline in concentration would be due to loss to the atmosphere. There is evidence in the literature, however, that native soil bacteria will degrade phenanthrene (Evans and et al 1965, Kiyohara and Nagao, 1978). The results of this study confirm that phenanthrene was relatively easily degraded in soil.

The phenanthrene half lives calculated, assuming first order kinetics for a 19-day period, were 9.7 and 14 days for the 5 and 50 $mg \cdot kg^{-1}$ treatments, respectively and were in the middle of the range reported in the literature (Table 8.1.2).

FIGURE 8.1.2 PHENANTHRENE DISAPPEARANCE FROM SOIL

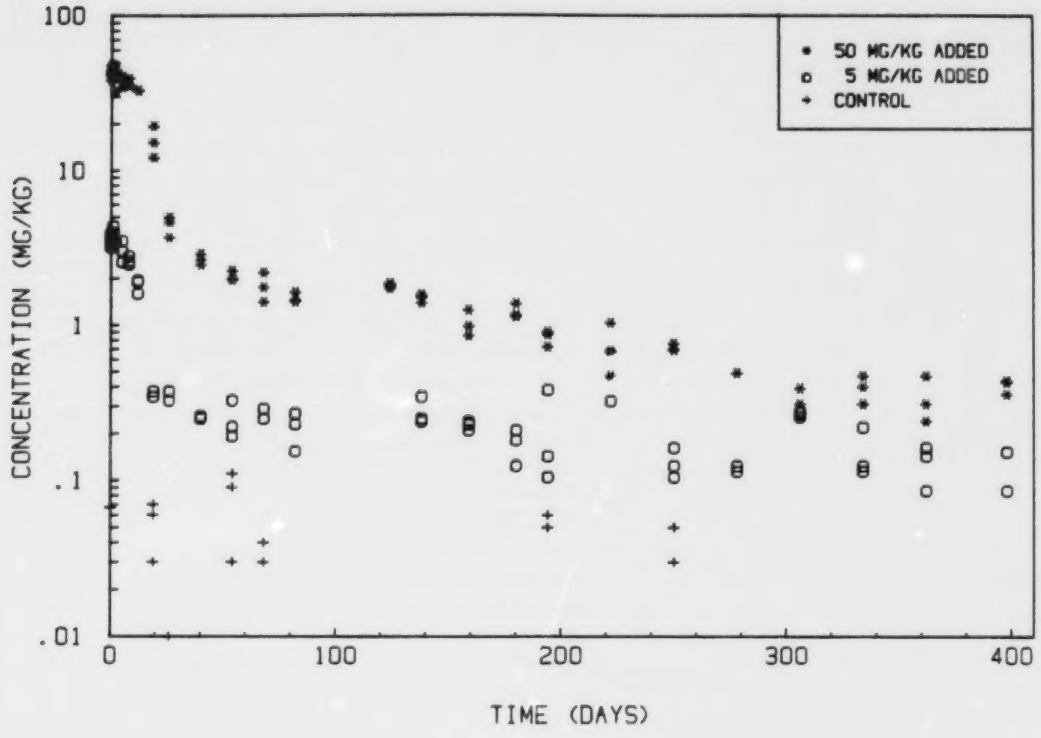
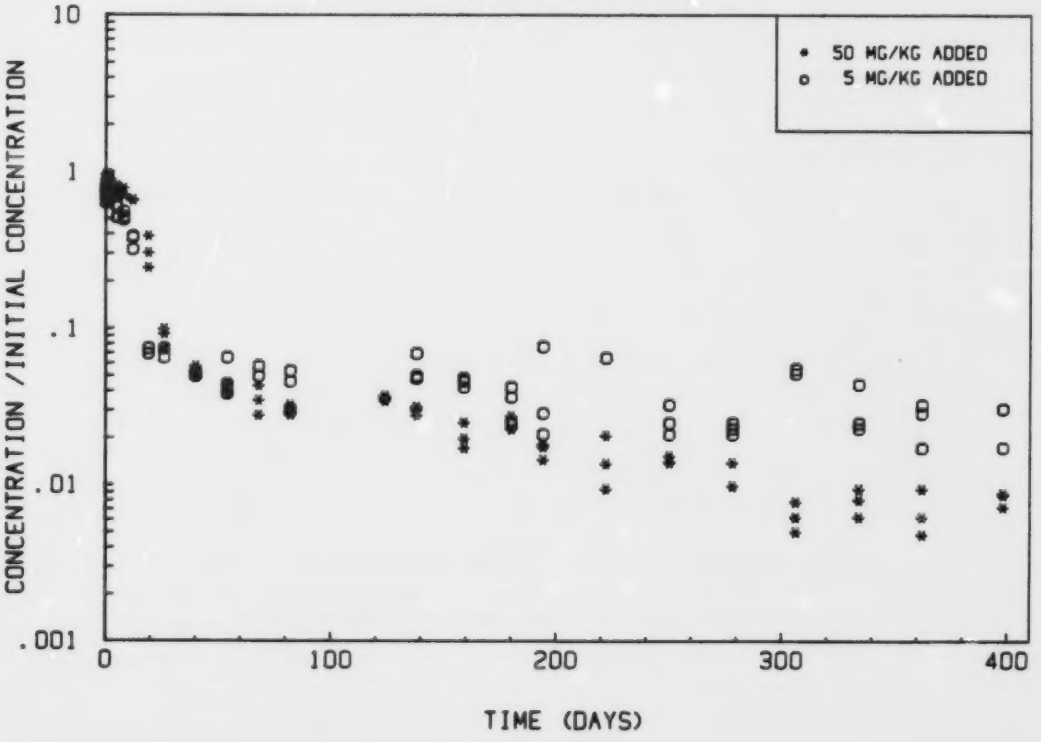


FIGURE 8.1.2a PHENANTHRENE DISAPPEARANCE FROM SOIL (CONCENTRATION/ INITIAL CONCENTRATION)



8.1.3 Anthracene

The concentration of anthracene was reduced by approximately 96% over a period of about 160 days, as illustrated in Figure 8.1.3 and an adaptation period of about 19 days was observed for the 50 $mg \cdot kg^{-1}$ treatment. The model which fit the data best over a 400-day period was one of 1.42 order with a rate constant of 0.0268 day^{-1}, and 1.31 order with a rate constant of 0.0097 day^{-1}, for the 5 and 50 $mg \cdot kg^{-1}$ treatments, respectively (Table 8.1.1). Alternatively, inspection of the data in Figure 8.1.3 suggested that two kinetic stages may have occurred.

In the case of anthracene the response differed with the two levels of application and the rate constant varied with the initial concentration. With the 50 $mg \cdot kg^{-1}$ treatment the decrease in anthracene concentration appeared to occur after an adaptation period and was slower than with the 5 $mg \cdot kg^{-1}$ treatment. Herbes and Schwall (1978) also noted a reduction in the degradation kinetics of anthracene with increased level of addition and suggested that it was due to saturation of interstitial water by anthracene, saturation of enzymatic pathways or toxic inhibition of microbial activity. The relatively low sorption coefficient and low solubility of anthracene suggests that soil water was saturated at the 50 $mg \cdot kg^{-1}$ treatment. In addition the total loading of PAH's at the 50 $mg \cdot kg^{-1}$ treatment was high and may have resulted in initial inhibition.

A reaction order of at least one is indicated for anthracene and suggests that the disappearance was concentration dependent, which is consistent with the decline of a microbial population with reduced availability of substrate.

Although anthracene has the same molecular weight as phenanthrene, it is less water soluble and may have been less available for microbial degradation, and therefore more persistent. Also, because of low water solubility, it has a high Henry's Law constant, relative to the other PAH's, and may volatilize. Half lives for anthracene predicted on a first order assumption (17 and 45 days) were greater than those reported by Herbes and Schwall (1978) with precontaminated freshwater sediment (11.5 days) but were low in comparison to those reported by Gardner et al (1979) with marine marsh sediment (175 days).

8.1.4 Fluoranthene

The concentration of fluoranthene in soil decreased after an adaptation period of approximately 19 days, as illustrated in Figure 8.1.4 Approximately 96% of the compound disappeared within 160 days. Regression of the data over a 400-day period indicated a best fit with a reaction order of 1.40 with a rate constant of 0.0223 day^{-1} for the 5 $mg \cdot kg^{-1}$ treatment and a reaction order at 1.37 with a rate constant of 0.0131 day^{-1} for the 50 $mg \cdot kg^{-1}$ treatment (Table 8.1.1). As suggested for anthracene and phenanthrene, however, the deviation of the estimated order from a first order reaction may be the result of two kinetic stages over the 400 day period. As noted for phenanthrene, the initial first order rate constant for fluoranthene (to approximately 160 days) appeared to be

FIGURE 8.1.3 ANTHRACENE DISAPPEARANCE FROM SOIL

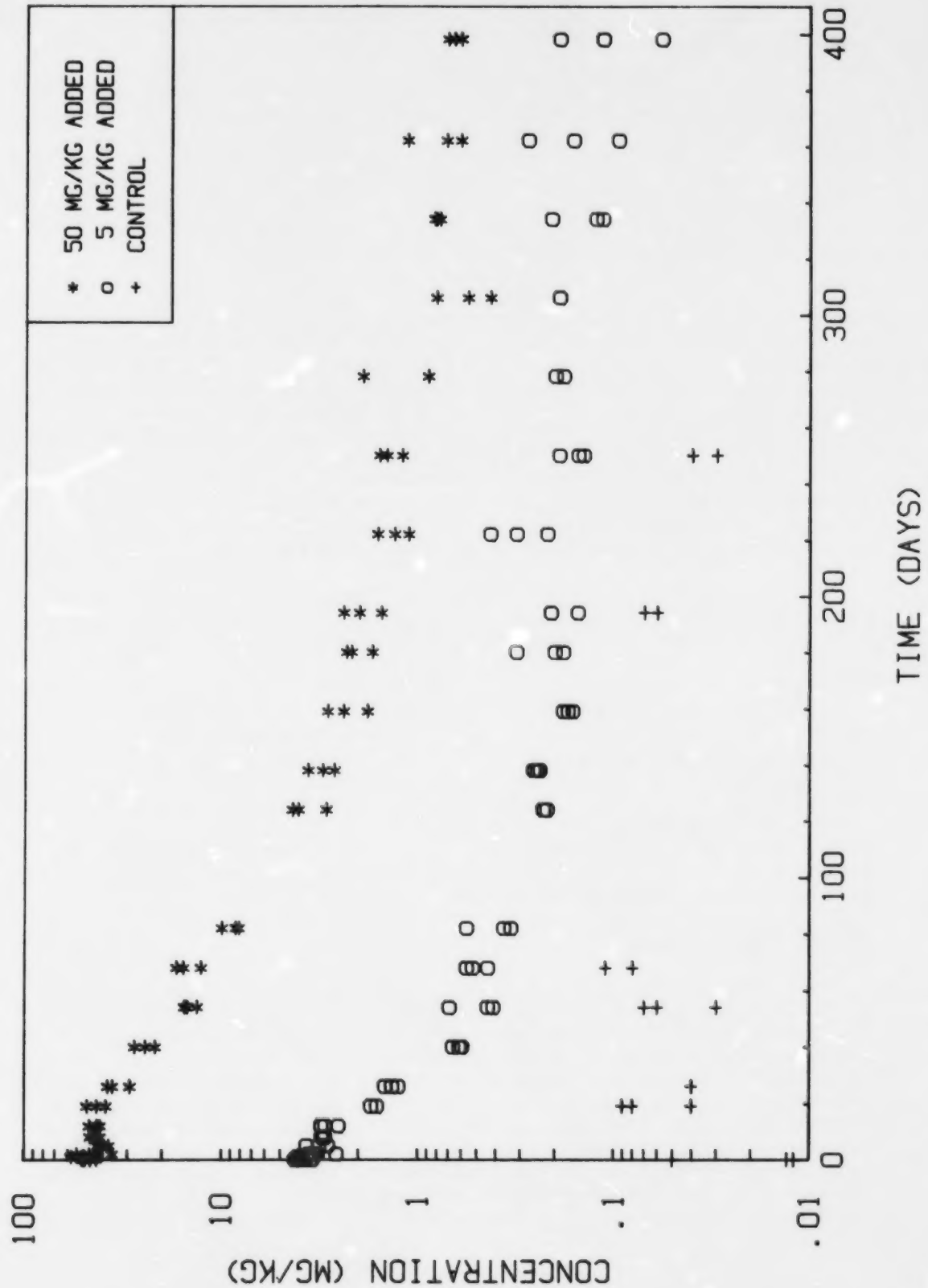


FIGURE 8.1.4 FLUORANTHENE DISAPPEARANCE FROM SOIL

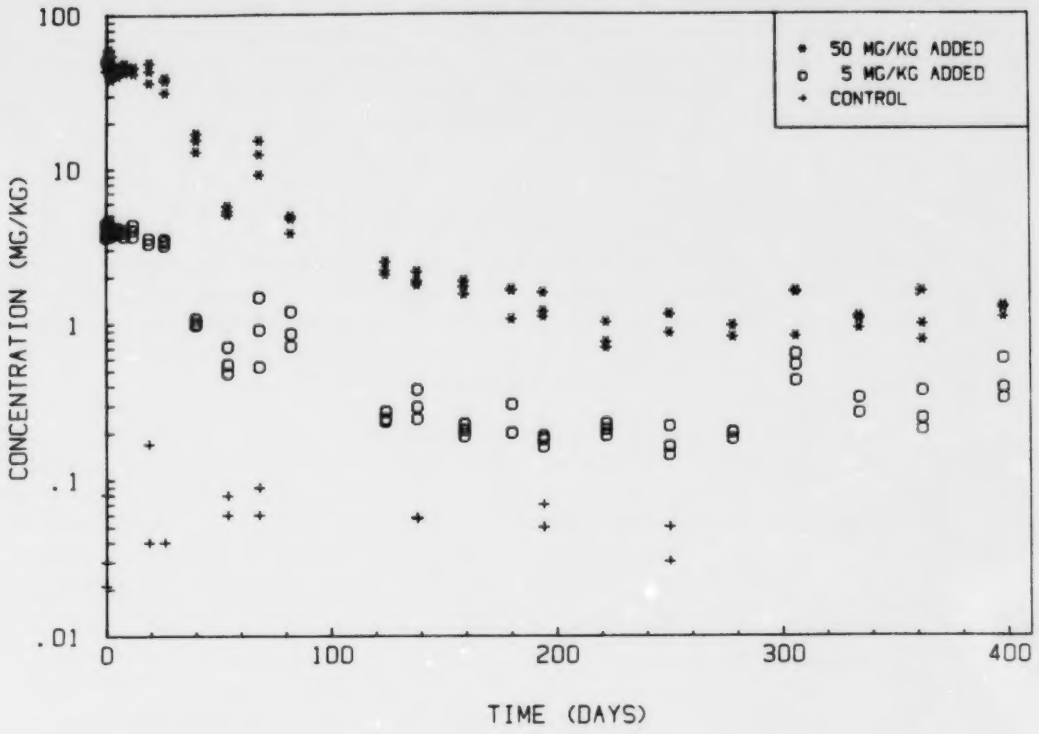
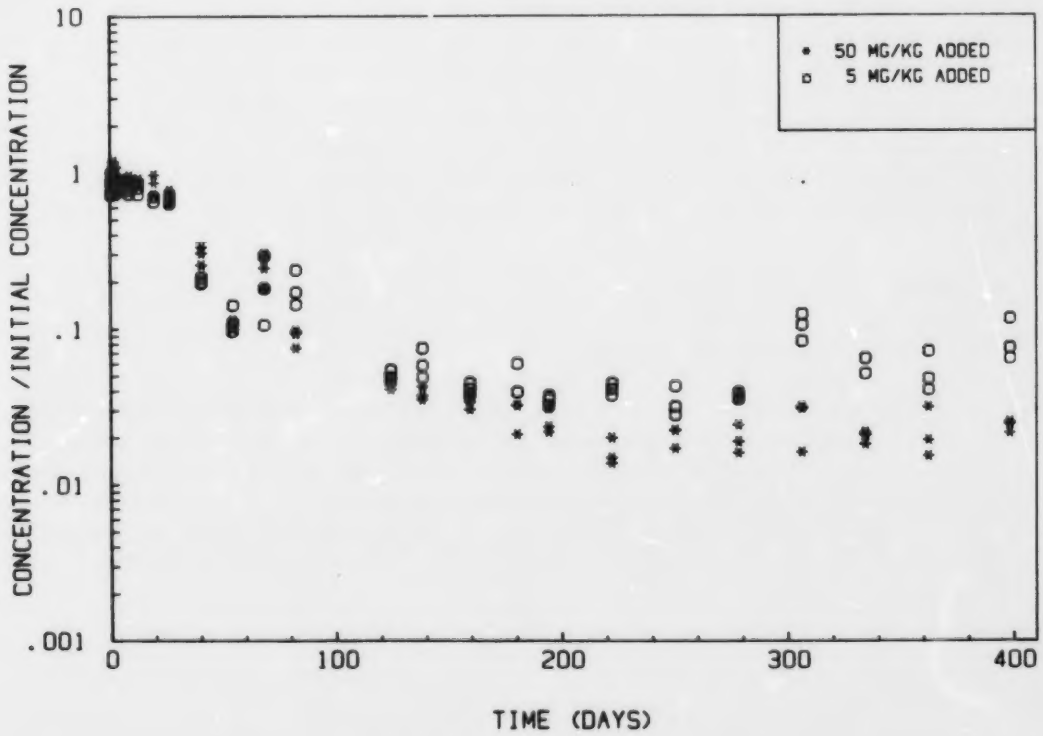


FIGURE 8.1.4a FLUORANTHENE DISAPPEARANCE FROM SOIL (CONCENTRATION/ INITIAL CONCENTRATION)



independent of the initial concentration, as illustrated by the normalized plot in Figure 8.1.4.a. After 160 days, however, the rate constant appeared to be less for the 5 $mg \cdot kg^{-1}$ treatment than for the 50 $mg \cdot kg^{-1}$ treatment. After approximately 200 days very little change in concentration occurred with either treatment.

The occurrence of an adaptation period followed by a reduction in concentration of at least first order suggests degradation by an adapted microbial population. Such a population would have achieved growth on the substrate during the adaptation phase but declined as the substrate was depleted. The decrease in rate of disappearance did not occur at a limiting concentration for the 50 $mg \cdot kg^{-1}$ treatment, however. Some other factor, such as changes in sorptive behaviour may have reduced the availability of fluoranthene for degradation processes.

Fluoranthene has a higher molecular weight than anthracene but is more soluble in water. Following the period of adaptation it appeared to disappear as quickly as anthracene. First order half lives (including 19 days for adaptation) were 39 and 34 days for the 5 and 50 $mg \cdot kg^{-1}$ levels of addition, respectively. These half lives were low in comparison to many values reported in the literature but similar to those reported by Groenewegen and Stolp (1976) (44 days).

8.1.5 Pyrene

Inspection of the data, illustrated in Figure 8.1.5, suggested that no change in the concentration of pyrene occurred for approximately 26 days. Approximately 96% of the pyrene disappeared, however, within 200 days. Regression analysis indicated that the best fit occurred with a reaction order of 1.04 and rate constant of 0.0180 day^{-1} with the 5 $mg \cdot kg^{-1}$ treatment and with an order of 1.08 and rate constant of 0.0220 day^{-1} with the 50 $mg \cdot kg^{-1}$ treatment and did not differ significantly from a first order model (Table 8.1.1). The initial first order constant for pyrene (to approximately 200 days) appeared to be independent of the initial concentration, as illustrated in Figure 8.1.5a. Following 200 days, the rate constant appeared to be lower with the 5 $mg \cdot kg^{-1}$ treatment than with the 50 $mg \cdot kg^{-1}$ treatment, but the pyrene data is variable and there was statistically no difference between the estimated rate constants. After approximately 250 days no change in pyrene concentration was evident.

The occurrence of an adaptation phase followed by a first order reaction suggested that degradation was performed by an adapted microbial population. After 200 days, however, some factor other than concentration limited degradation processes.

Pyrene and fluoranthene are similar in molecular weight and physical properties and appeared to be degraded at a similar rate in soil. First order half lives of approximately 58 and 48 days (including 26 days of adaptation period) were calculated for pyrene at the 5 and 50 $mg \cdot kg^{-1}$ treaments, respectively. These values were somewhat high in comparison to the range of values reported by Sims and Overcash (1983) (Table 8.1.2).

FIGURE 8.1.5 PYRENE DISAPPEARANCE FROM SOIL

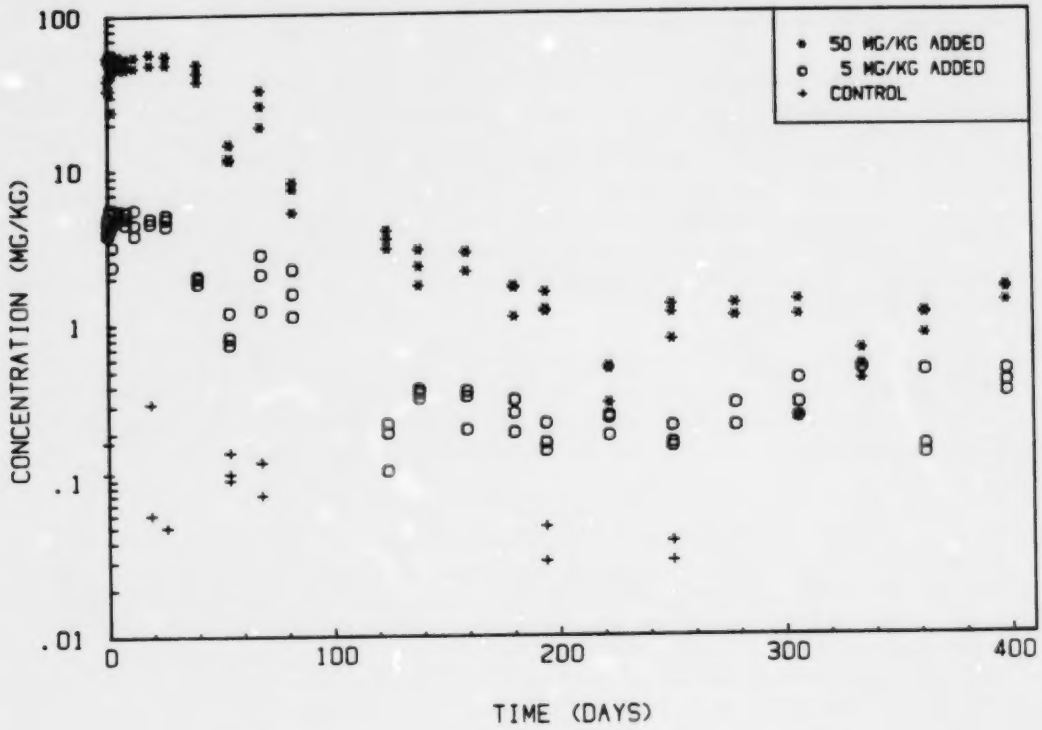
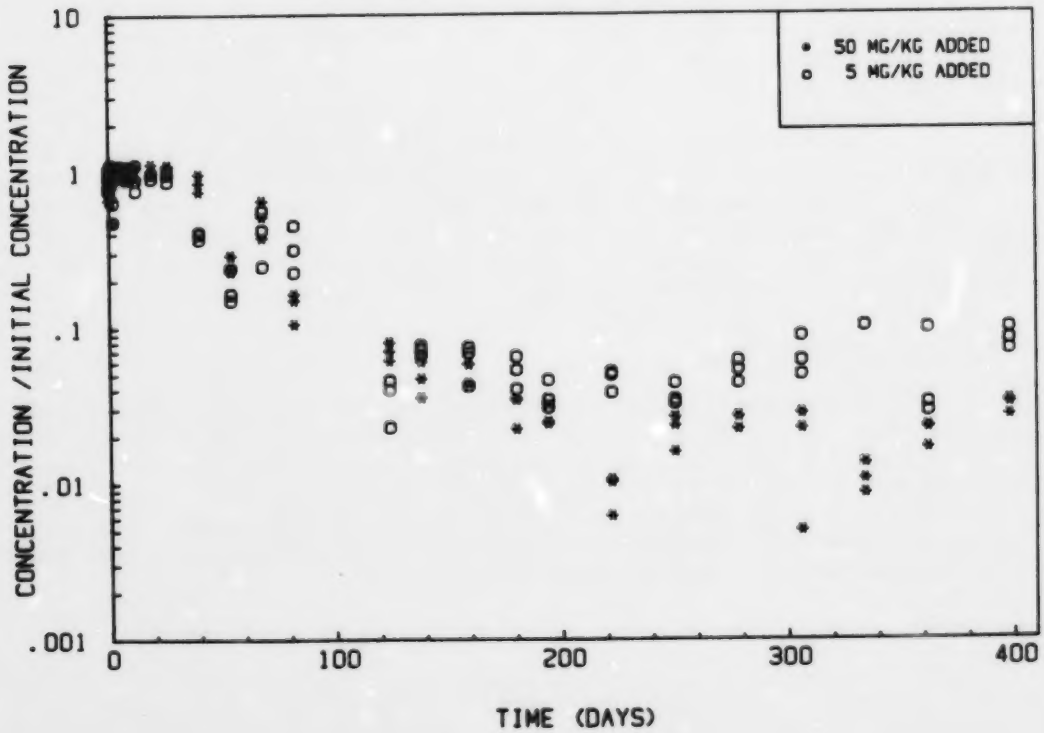


FIGURE 8.1.5a PYRENE DISAPPEARANCE FROM SOIL (CONCENTRATION/ INITIAL CONCENTRATION)



8.1.6 Benzo(a)anthracene

The degradation of benzo(a)anthracene appeared to occur after an adaptation phase of about 40 days, as illustrated in Figure 8.1.6. With the 5 $mg \cdot kg^{-1}$ treatment the best fit by non-linear regresson analysis was with a reaction order of 2.72 and rate constant of 0.0006 day^{-1} (Table 8.1.1). Inspection of the data in Figure 8.1.6, however, suggested that a first order model (rate constant 0.0034 day^{-1} ∓15%) may be appropriate. The concentration of benzo(a)anthracene declined slowly (about 60% in 400 days) with considerable variability in the data, making discrimination between models difficult.

With the 50 $mg \cdot kg^{-1}$ treatment the model fit with a reaction order of 1.00 and rate constant of 0.0080 day^{-1}, which was supported by the straight line logarithmic plot of the data. The concentration of benzo(a)anthracene decreased by about 88% in 400 days with the 50 $mg \cdot kg^{-1}$ treatment.

With both levels of benzo(a)anthracene addition, the activity of an adapted microbial population was suggested by the presence of an adaptation phase followed by a decrease in concentration with at least first order kinetics. The difference in rate constants for the two treatments may indicate that degradation processes are enhanced above a certain concentration, for example through the stimulation of enzymatic pathways. Benzo(a)anthracene is similar to anthracene in that the initial first order rate constants differ for the two levels of PAH addition. Degradation of benzo(a)anthracene had not yet proceeded to the point where results from the two treatments could be compared for the same concentration remaining in soil.

Benzo(a)anthracene is a high molecular weight PAH, less water soluble than those discussed previously and more inclined to adsorb to soil surfaces. These properties may have made it less accessible for biological or chemical degradation.

On the assumption of a first order reaction, the half lives of benzo(a)anthracene, including 40 days for adaptation, were approximately 240 and 130 days for the 5 and 50 $mg \cdot kg^{-1}$ treatments. These half lives are within the range of values reported by Sims and Overcash (1983) and are similar to those reported by Gardner et al (1979) for marine marsh sediment (252 days) and Groenewegen and Stolp (1976) for soil (102 days).

8.1.7 Chrysene

The concentration of chrysene in soil decreased very slowly as illustrated in Figure 8.1.7. A zero order model (linear decrease with time) provided the best fit to the data with both the 5 and 50 $mg \cdot kg^{-1}$ treatments with rate constants of 0.0081 and 0.1179 day^{-1}, respectively (Table 8.1.1). As very little change in chrysene concentration occurred with the 5 $mg \cdot kg^{-1}$ treatment, confidence intervals were wide and the regression analysis was not very conclusive.

FIGURE 8.1.6 BENZO(A)ANTHRACENE DISAPPEARANCE FROM SOIL

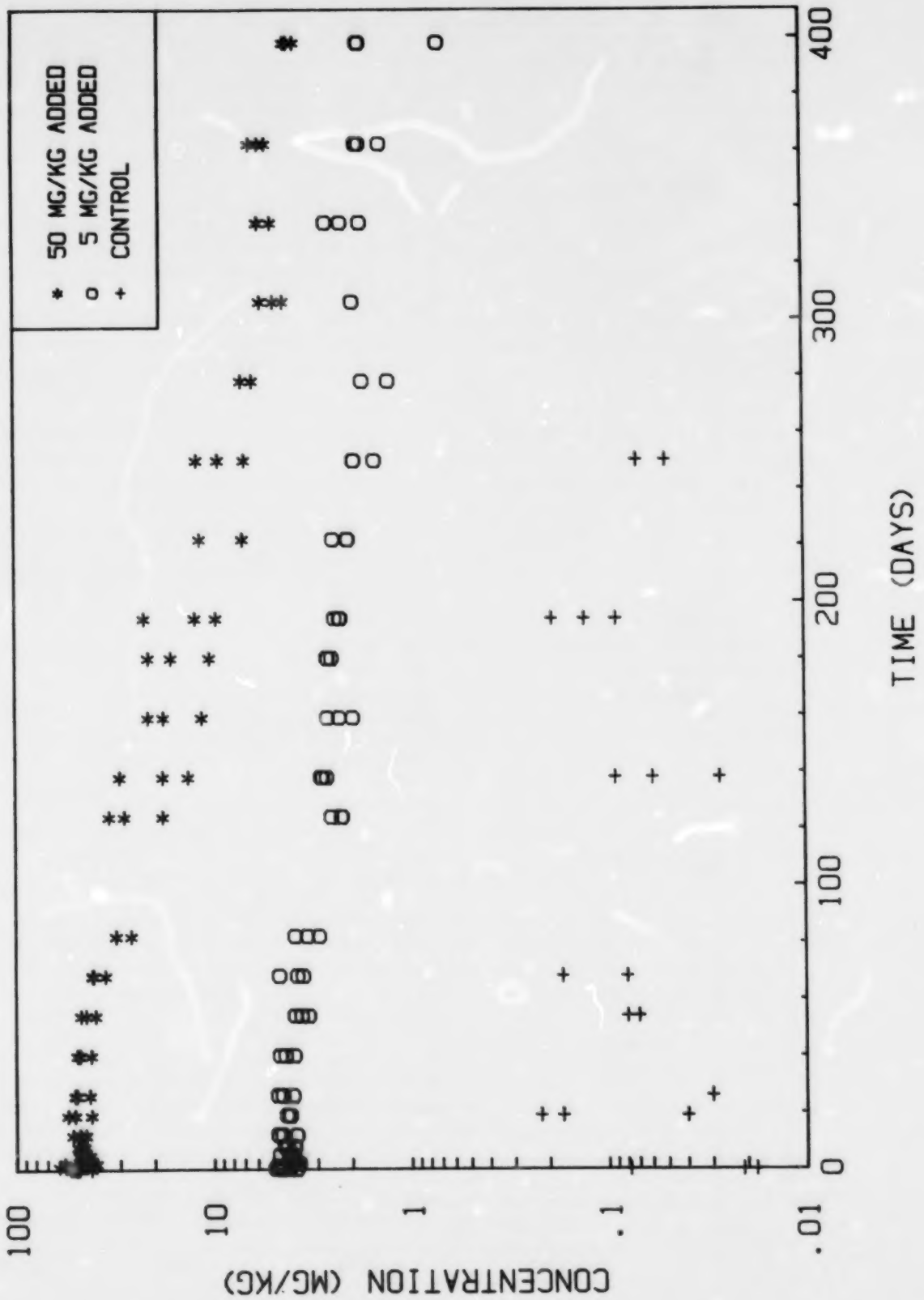


FIGURE 8.1.7 CHRYSENE DISAPPEARANCE FROM SOIL

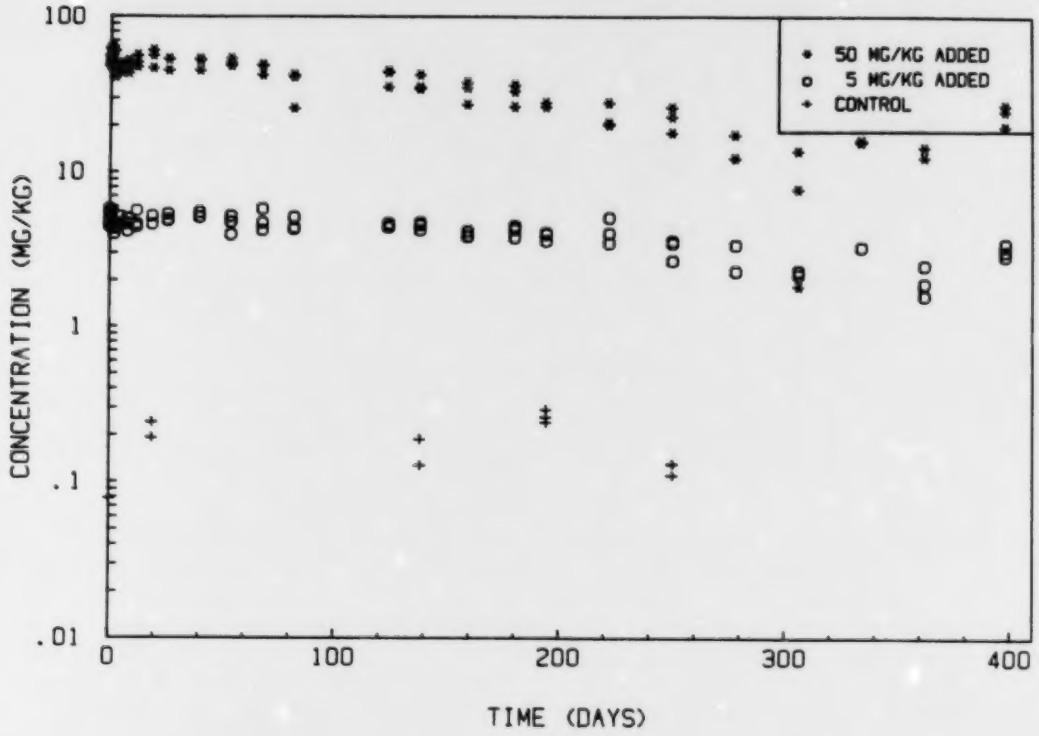
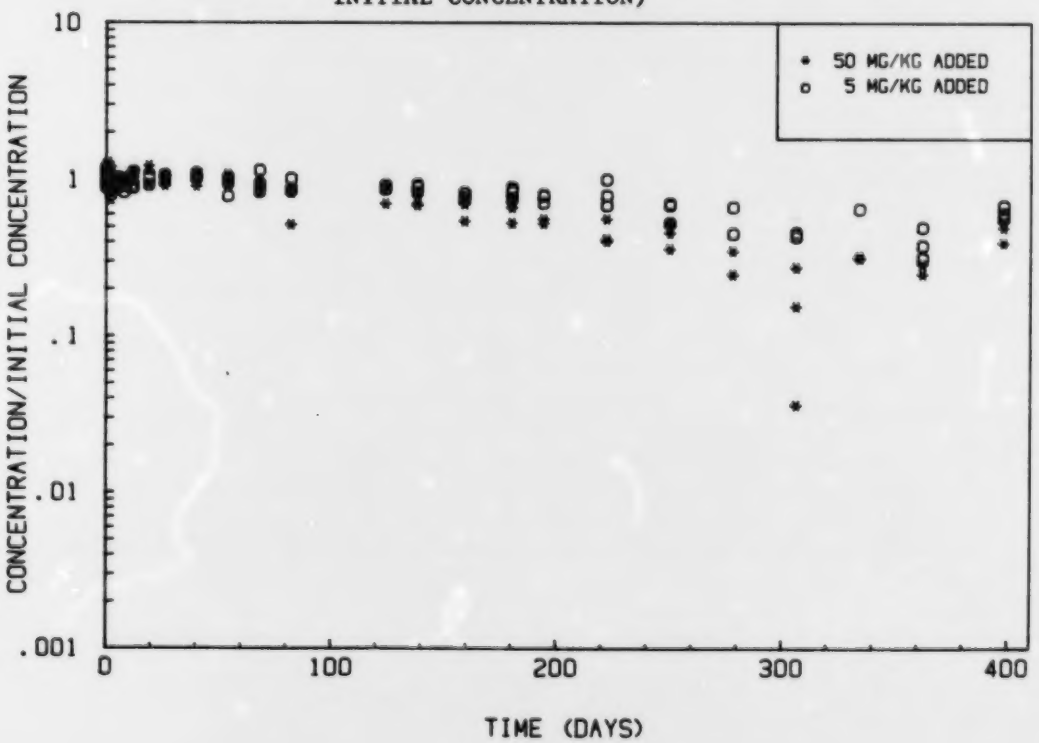


FIGURE 8.1.7a CHRYSENE DISAPPEARANCE FROM SOIL (CONCENTRATION/ INITIAL CONCENTRATION)



The zero order decrease in concentration suggested that adaptation and growth of a specific microbial population was not involved. Degradation may have occurred randomly by the microbial population at large, or may have proceeded as a result of physical or chemical reactions. Degradation was more rapid with the 50 $mg \cdot kg^{-1}$ treatment than with the 5 $mg \cdot kg^{-1}$ treatment, possibly because there was more chrysene available in the soil water for reaction.

The first order rate constants appeared to be independent of the initial concentration, as illustrated in Figure 8.1.7a. Results from the 5 and 50 $mg \cdot kg^{-1}$ treatments could not be compared, however, for the same concentration remaining in soil.

Chrysene has the lowest water solubility and highest sorption coefficient of the PAH's studied and these properties likely limited its availability for reaction.

The degradation of chrysene reported in the literature is typically slow and certain authors (Groenewegen and Stolp, 1976) have reported no degradation at all. Half lives of approximately 328 and 224 (zero order) were predicted from the results of this study for 5 and 50 $mg \cdot kg^{-1}$ of chrysene addition. Clearly lengthy incubation times are required for assessment of the degradation of chrysene.

8.1.8 Benzo(a)pyrene

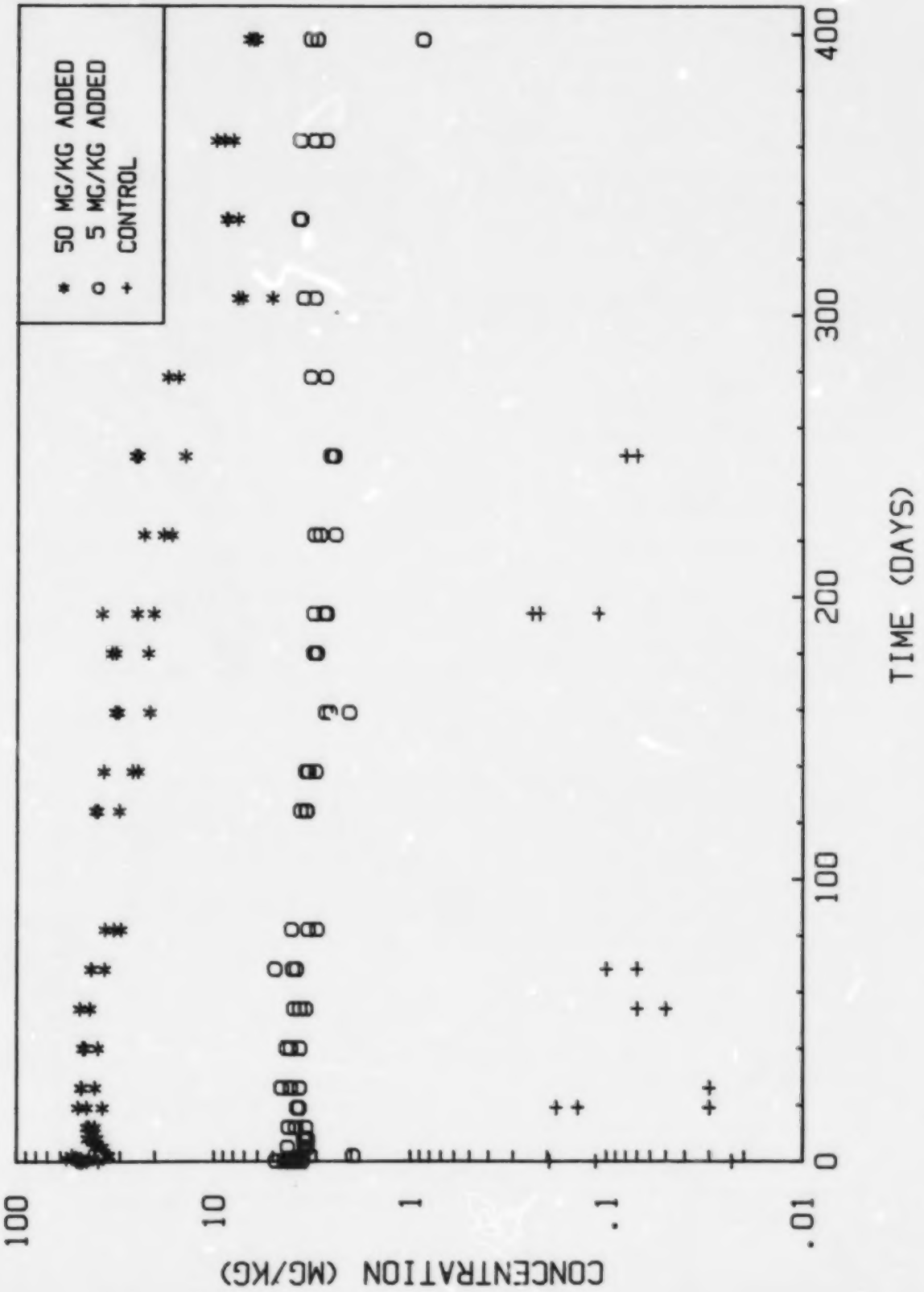
The concentration of benzo(a)pyrene in soil also decreased slowly (Figure 8.1.8). Non-linear regression indicated a best fit with a model of 1.01 order and rate constant of 0.0009 day^{-1} for the 5 $mg \cdot kg^{-1}$ treatment, however the decrease in concentration was small (about 22% in 400 days) and variability was high so it was not possible to discriminate between models (Table 8.1.1). A zero order model (rate constant 0.0058 day^{-1} ∓42%) may also describe the data.

Greater precision was obtained in the estimation of reaction order for the 50 $mg \cdot kg^{-1}$ treatment because a greater change in concentration occurred (87% in 400 days). A zero order model provided the best fit to the data with a rate constant of 0.1031 day^{-1}.

Degradation of benzo(a)pyrene did not appear to be associated with the growth of a specific microbial population. A zero order kinetic model may indicate that degradation was performed by an unadapted soil microbial community, or through chemical or physical degradation processes. Like benzo(a)anthracene, the first order rate constant varied with the initial concentration. The rate constant was greater with the 50 $mg \cdot kg^{-1}$ treatment than with the 5 $mg \cdot kg^{-1}$ treatment, possibly because degradation processes were stimulated by the higher concentration. Degradation had not yet proceeded to the point where results from the 5 $mg \cdot kg^{-1}$ and 50 $mg \cdot kg^{-1}$ treatments could be compared for the same concentration remaining in soil.

The persistence of benzo(a)pyrene may have been due to its high molecular weight, low water solubility and high sorptivity to soil.

FIGURE 8.1.8 BENZO(A)PYRENE DISAPPEARANCE FROM SOIL



Some studies have reported no degradation of benzo(a)pyrene in soil (Shabad et al 1971). The half lives predicted in this study (approximately 347 and 218 days at the 5 and 50 $mg \cdot kg^{-1}$ treatments, respectively) are similar to those reported by Gardner et al (1979) (420 days) for marine marsh sediment.

8.1.9 Implications for the Land Treatment of Oily Wastes

The rates of disappearance observed were generally in agreement with observations made in other studies with a range of soils or sediments. Several PAH's disappeared rapidly from soil and for some PAH's the rates of disappearance were greater than observed for other workers, indicating that the incubation conditions were adequate for assessing PAH disappearance. The results of this study are specific for the addition of pure PAH's to Donnybrook sandy loam and incubated under specific conditions. In soil treated with oily waste, the rates of PAH disappearance may be different. This study does, however, give an indication of the degradability of PAH's in a soil environment. It indicates the time period required for adequate study, the usefulness of certain kinetic models, the sampling variability encountered, and potential concerns which should be addressed when studying the persistence of PAH's in soil and waste matrices.

Low rates of disappearance were generally associated with PAH's of high molecular weight and sorption coefficients and low water solubilities. Assuming the rates of disappearance observed for PAH loadings in this study, naphthalene would not be expected to persist in soil used for land treatment of oily wastes. Other low to medium molecular weight PAH's persisted at concentrations above background levels (0.1 to 0.5 $mg \cdot kg^{-1}$) when applied at 50 $mg \cdot kg^{-1}$. Assuming the rates of disappearance observed in this study (99.6% for phenanthrene and 98% for anthracene, fluoranthene and pyrene in one year) annual loadings in excess of 25 $mg \cdot kg^{-1}$ (approximately 50 $kg \cdot ha^{-1}$) for phenanthrene and 5 $mg \cdot kg^{-1}$ (10 $kg \cdot ha^{-1}$) for anthracene, fluoranthene and pyrene in soil may result in accumulation of these PAH's with time.

Bossert et al (1984) studied an oil refinery waste with concentrations of 379 mg phenanthrene $\cdot kg^{-1}$ and 79.4 mg anthracene $\cdot kg^{-1}$ (wet weight basis). According to the estimate above, sludge with these concentrations would be limited to annual applications of 126 tonnes $\cdot ha^{-1}$ (wet weight) to prevent anthracene accumulation with time (i.e., 10 $kg \cdot ha^{-1}$/79.4 $mg \cdot kg^{-1}$). High molecular weight PAH's are present in refinery wastes at much lower concentrations. The waste used by Bossert et al (1984) contained 10.3 $mg \cdot kg^{-1}$ benzo(a)pyrene and 11.3 $mg \cdot kg^{-1}$ chrysene. At 126 tonnes $\cdot ha^{-1}$, sludge application would result in addition of 0.63 $mg \cdot kg^{-1}$ benzo(a)pyrene to soil on an annual basis. Due to the persistence of these PAH's, however, such small additions may lead to accumulations in soil. The results of this study indicate that annual applications as low as 5 $mg \cdot kg^{-1}$ of benzo(a)anthracene, chrysene and benzo(a)pyrene in soil would result in accumulation with time. Based on degradation rates of 22 and 88% (worst and best cases in this study), annual application in excess of 0.26 and 1.67 $mg \cdot kg^{-1}$ respectively, may result in accumulation in soil. The results of Part I suggest that,

depending on the PAH content of oily sludges, application rate and frequency, accumulation of PAH's may occur in soil used for land treatment.

Studies using oily waste and soil mixtures are required to adequately assess the persistence of PAH's in oily wastes applied to land. Such a study requires at least triplicate samples and should proceed until the concentration in soil approaches the background concentration. This process may be time consuming, requiring incubation in excess of one year, but extrapolation from data collected early in the incubation period is likely to result in a poor estimate of persistence.

8.2 Part II - ^{14}C Studies

The fate of benzo(a)pyrene and anthracene in the soil-biometer flask system was studied by measuring the distribution of ^{14}C in the system following addition of ^{14}C labelled chemicals. The amounts of ^{14}C measured in the various components of the biometer flask system have been corrected for background ^{14}C and summarized in Tables 8.2.1 and 8.2.2 for ^{14}C-benzo(a)pyrene and ^{14}C-anthracene, respectively.

In these tables, the soil extract in hexane and soil extract in water refer to the fractions of ^{14}C in soil extract which were partitioned into hexane and water, respectively. Soil residue refers to ^{14}C which was not extracted from soil, and therefore considered irreversibly bound (Khan, 1982). This material may include parent PAH and/or metabolites. Parent PAH which has become bound to soil solids may not have undergone a chemical reaction, but has become physically separated from extractable PAH and for purposes of discussion is considered to have reacted with soil. The polyurethane plug refers to ^{14}C which was emitted from soil as volatile hydrocarbons (parent PAH or metabolites) while CO_2 refers to the portion of ^{14}C which was emitted as the final degradation product.

Data is included in the tables for the 5 and 50 $mg \cdot kg^{-1}$ application rates of PAH with sterilized (5000 $mg \cdot kg^{-1}$ $HgCl_2$) and biologically active (0 $mg \cdot kg^{-1}$ $HgCl_2$) soil. Treatment means were compared for each component of the system by analysis of variance and Duncan's multiple range test. Means in a column which are followed by the same letter were not significantly different at a 95% confidence level. A detailed discussion of each table follows.

8.2.1 Benzo(a)pyrene

The distribution of ^{14}C from ^{14}C-benzo(a)pyrene in the various components of the biometer flask system after 145 days have been summarized in Table 8.2.1. The total amount of ^{14}C recovered from the flasks ranged from 72 to 82% and was typical for a ^{14}C tracer study. Some ^{14}C may be lost during analysis, for example, by adsorption to glassware.

The hexane fraction of the soil extract contained the majority of ^{14}C recovered from the flasks. All ^{14}C found in the hexane was confirmed as benzo(a)pyrene by TLC. An average of 70% of added benzo(a)pyrene was thus recovered as parent compound. Recovery appeared to be lower with the 5 $mg \cdot kg^{-1}$ treatment than with the 50 $mg \cdot kg^{-1}$ treatment. The variance

was high, however, due to one low value with the 5 $mg \cdot kg^{-1}$ benzo(a)pyrene, 5000 $mg \cdot kg^{-1}$ $HgCl_2$ treatment, and the difference between application levels was not statistically significant. There was no significant effect of $HgCl_2$ addition on the amount of ^{14}C recovered in hexane. Thus benzo(a)pyrene, as recovered by the analytical procedure, did not appear to be affected by biological activity.

An average of only 8% of ^{14}C was recovered as material that interacted with soil. The water fraction of the soil extract (water soluble metabolites) accounted for a small proportion of the reaction products and the effects of PAH concentration or biological activity were

TABLE 8.2.1 THE PERCENT OF ^{14}C RECOVERED IN EACH COMPONENT OF THE BIOMETER FLASK SYSTEM AFTER 145 DAYS WITH TWO LEVELS OF BENZO(A)PYRENE (BAP) AND $HgCl_2$ TREATMENT

TREATMENTS		COMPONENT OF SYSTEM					TOTAL
BAP $mg\ kg^{-1}$	$HgCl_2$ $mg\ kg^{-1}$	Soil Extract in Hexane	Soil Extract in Water	Soil Residue	Poly-urethane Plug	CO_2	RECOVERED
5	0	70.0 a	0.02 a	9.6 b	0.17 b	0.059 b	79.9
50	0	78.1 a	0.18 a	4.6 a	0.19 b	0.010 ab	83.1
5	5000	59.5 a	0.38 a	12.7 b	0.05 a	0.012 ab	72.6
50	5000	72.6 a	0.12 a	4.6 a	0.08 ab	0.003 a	77.4

[1]Values within a column which are followed by the same letter are not significantly different as determined by Duncan's multiple range test at a 95% confidence level.

not significant. The ^{14}C found in the water fraction was not likely benzo(a)pyrene because its concentration greatly exceed the water solubility of benzo(a)pyrene.

The amount of ^{14}C in soil residue after extraction represented the greatest proportion of reaction products. This residual fraction may have been bound to soil through physical or chemical action. As the proportion of ^{14}C in soil residue was at least as high with 5000 as with 0 $mg \cdot kg^{-1}$ $HgCl_2$ treatment, ^{14}C was not likely incorporated into soil biomass through microbial activity. The proportion of residual ^{14}C was reduced at the high rate of benzo(a)pyrene addition but there was insufficient information to define sorption or binding relationships.

Very small proportions of ^{14}C were recovered from the polyurethane plug (volatilized benzo(a)pyrene or its metabolites) and from NaOH (CO_2). These fractions were slightly reduced by $HgCl_2$ treatment (the main effect of $HgCl_2$ on ^{14}C was significant) suggesting, particularly in the case of CO_2, that some microbial interaction with benzo(a)pyrene occurred. Relative to the total amount of benzo(a)pyrene that was reacted, microbial degradation appeared to be negligible. The overall biological

activity of the soil was assessed by measuring total CO_2 which was trapped in NaOH and analysed by titration. Evolution of CO_2 averaged 0.02 $mg \cdot g^{-1} soil \cdot day^{-1}$ in the soil without $HgCl_2$ addition and was considered a normal level of biological activity for unamended agricultural soil. Total CO_2 evolution was not affected by PAH addition.

Although the proportions of ^{14}C found as reaction products were small, the evidence suggests that what reaction did occur was predominantly the result of physical or chemical processes.

The results of the ^{14}C study concur with the results in Part I in that disappearance of benzo(a)pyrene was slow. Loss of benzo(a)pyrene in the hexane fraction was less in Part II than in Part I. The presence of other PAH's incubated with soil in Part I may have stimulated the degradation of benzo(a)pyrene, for example, through co-metabolism or some other synergistic effect. There was not enough data collected in the ^{14}C study to define orders of reaction. Zero order models fit the extractable benzo(a)pyrene data with rate constants of 0.108 (∓91%) and 0.0926 (∓155%) for the 5 and 50 $mg \cdot kg^{-1}$ treatments. The confidence limits are wide and reflect limited data, however the estimates compare favourably with the results for the 50 $mg \cdot kg^{-1}$ treatment in Part I.

The proportions of ^{14}C recovered as reaction products in sacrifice flasks generally supported the results from flasks incubated the full 145-day period. Approximately 0.0 to 0.9% was recovered in the water extract, 2 to 32% in soil residue and 0.01 to 0.30% on the polyurethane plug. These samples were not replicated, however, and no consistant trend with time was evident. The data was too limited to determine if the production of water soluble metabolites, for example, was of greater significance earlier in the incubation.

8.2.2 Anthracene

Total recovery of ^{14}C was similar for anthracene as for benzo(a)pyrene. Recovery was also consistently lower with the 5 $mg \cdot kg^{-1}$ treatment than with the 50 $mg \cdot kg^{-1}$ treatment. The distribution of ^{14}C measured in the components of the biometer flask system have been summarized for anthracene in Table 8.2.2 and are detailed below.

Most of the ^{14}C recovered from the flasks was found in the hexane fraction of the soil extract and was confirmed as anthracene by TLC. This fraction was significantly lower with the 5 $mg \cdot kg^{-1}$ treatment than with the 50 $mg \cdot kg^{-1}$ treatment in both sterilized and biologically active soil. This observation is in agreement with the results of Part I, in which the concentration of anthracene, as measured in the hexane fraction of soil extract, decreased faster with the 5 $mg \cdot kg^{-1}$ treatment than with the 50 $mg \cdot kg^{-1}$ treatment. This difference was apparently not dependent on biological activity.

Little ^{14}C was found in the water fraction of the soil extract. Polar metabolites may have been quickly degraded volatilized or sorbed to soil solids. The effects of PAH concentration or biological activity were not consistent.

TABLE 8.2.2 THE PERCENT OF ^{14}C RECOVERED IN EACH COMPONENT OF THE BIOMETER FLASK SYSTEM AFTER 91 DAYS WITH TWO LEVELS OF ANTHRACENE (ANTH) AND $HgCl_2$ TREATMENT

TREATMENTS		COMPONENT OF SYSTEM					TOTAL
ANTH mg kg^{-1}	$HgCl_2$ mg kg^{-1}	Soil Extract in Hexane	Soil Extract in Water	Soil Residue	Poly-urethane Plug	CO_2	RECOVERED
5	0	51.1 a[1]	0.200 b	8.9 bc	14.6 c	.083 c	74.9
50	0	68.9 b	0.300 b	3.8 a	4.00 a	.057 b	77.1
5	5000	52.5 a	0.213 b	10.5 c	8.70 b	.005 a	71.9
50	5000	71.1 b	0.147 a	7.4 b	4.32 a	.005 a	83.0

[1]Values within a column which are followed by the same letter are not significantly different as determined by Duncan's mutliple range test at a 95% confidence level.

Binding to soil solids appeared to be an important mechanism affecting the fate of anthracene in soil. The proportion of ^{14}C bound to solids was greater with the 5 mg·kg^{-1} anthracene treatment than with the 50 mg·kg^{-1} treatment, as was observed for benzo(a)pyrene. Binding of ^{14}C was also as great in the sterilized soil as in the biologically active soil and therefore did not appear to be biologically mediated.

Volatilization also appeared to play an important role in the removal of anthracene from soil, as indicated by the collection of ^{14}C on the polyurethane plug. The proportion of ^{14}C collected on the plug was greater with the low level of anthracene addition than with the high level. Volatilization with the low level of anthracene was further enhanced in the biologically active soil, suggesting that some volatilization of biological metabolites occurred. The fact that substantial amounts of ^{14}C were volatilized suggests that the low overall recovery of ^{14}C was, in part, due to volatilization.

The proportion of ^{14}C collected as CO_2 was also significantly enhanced in the biologically active soil. The amount of ^{14}C that was collected as CO_2 was small, however, relative to the amount of ^{14}C that reacted. On the anthracene molecule, the ^{14}C label was on the 9 position (middle aromatic ring), and thus would be evolved as CO_2 following complete degradation of one of the end rings. It is possible, therefore, that partial degradation took place which did not result in liberation of ^{14}C as CO_2 but did result in bound or volatilized ^{14}C. Total CO_2 evolution (by titration) was similar to that in the ^{14}C-benzo(a)pyrene study (0.02 mg·g^{-1} soil·day^{-1}).

It was apparent that at least some of the mechanisms affecting removal of anthracene from soil were biologically mediated. The decrease in concentration observed in a soil extract, however, may reflect binding or volatilization of anthracene, rather than biological degradation. Care must be taken, therefore, in the interpretation of results from soil extracts alone.

9 CONCLUSIONS AND RECOMMENDATIONS

The results of this study showed that a detailed assessment of reaction kinetics was justified for several PAH's and that the assumption of a first order reaction was not always valid. In such cases, non-linear regression modelling was a useful technique for evaluating the kinetics of disappearance. For situations in which the change in concentration proceeded slowly, however, discrimination between models was not possible, even with replication and numerous sampling periods.

Reaction orders that were identified were approximately 0, 1 or between 1 and 2, depending on the PAH compound. **The use of a first order model to predict disappearance from soil, as is often performed, was clearly not adequate for some PAH's and, according to the data observed in this study, will underestimate residual PAH concentrations.**

Naphthalene, phenanthrene, anthracene, pyrene and fluoranthene initially disappeared rapidly from soil during a period of 200 days or less. A loss of 94 to 98% occurred during this initial period and approximated first order kinetics, in some cases following a lag period. Within this initial period, with the exception of anthracene, the first order kinetic rate constants were the same for 5 and 50 $mg \cdot kg^{-1}$ additions of PAH. Following the initial period, the remaining 2-6% of the added PAH was lost at a much reduced rate and the first order rate constants tended to be higher with the 50 $mg \cdot kg^{-1}$ addition than the 5 $mg \cdot kg^{-1}$ addition of PAH. The difference in rate constants for the two levels of addition may result from inhibition (in the case of anthracene) or stimulation of activity with the high PAH loadings. The change in concentration with time was not related to concentration alone, however, and may have been affected by other factors such as microbial population changes or changes in adsorption mechanisms. **For predictive purposes, either a model other than first order, or a combination of two models was required to adequately describe the loss of 99% of the added PAH.**

Losses of only 22 to 88% were observed for benzo(a)anthracene, chrysene and benzo(a)pyrene and only one kinetic stage was identified within the 400 day incubation period. With chrysene the first order kinetic rate constants were the same at the 5 and 50 $mg \cdot kg^{-1}$ levels of addition. For benzo(a)anthracene and benzo(a)pyrene the rate constants differed. The disappearance of benzo(a)anthracene approximated first order kinetics, but a zero order model was generally appropriate for the disappearance of benzo(a)pyrene and chrysene under the experimental conditions. **In the case of chrysene and benzo(a)pyrene, indiscriminate use of a first order model to predict the concentration in soil may seriously underestimate their persistence.**

The mechanisms of disappearance of anthracene and benzo(a)pyrene were assessed using ^{14}C labelling. The results indicated that biological activity was responsible for some of the loss of anthracene from soil. **Binding to soil solids and volatilization (either as anthracene or as metabolites) were identified as the major loss mechanisms for anthracene.** Identification of loss mechanisms for benzo(a)pyrene was less successful due to the small amount of benzo(a)pyrene that reacted within the incubation period. **Binding of benzo(a)pyrene to soil solids appeared to be the major mechanisms involved and microbial transformation of the**

compound was minimal. The observed tendency for ^{14}C to be bound or volatilized may be expected for hydrophobic chemicals such as anthracene and benzo(a)pyrene in a soil/water system. Chemical or biological metabolites may also favor these mechanisms.

The information from this study generally agrees with observations in the literature and provides more detailed information on the disappearance kinetics and mechanisms than was previously available for some PAH's. **The results are specific, however, for the addition of pure PAH's added to Donnybrook sandy loam and incubated under the specified conditions.** As a result the application of these results directly to land treatment situations may not be valid. Different rates of PAH disappearance may be observed with acclimated soil, different environmental matrices, soil types and field conditions. Furthermore, the implications for the environment of mechanisms such as soil binding are not known. Nonetheless, some general inferences can be drawn which may apply to land treatment practices.

Low rates of disappearance were associated with PAH's of high molecular weight and sorption coefficients and low water solubilities. Assuming the rate of disappearance observed in this study, annual loadings in excess of 25 $mg \cdot kg^{-1}$ (approximately 50 $kg \cdot ha^{-1}$) for phenanthrene and 5 $mg \cdot kg^{-1}$ (10 $kg \cdot ha^{-1}$) for anthracene, fluoranthene and pyrene in soil may result in accumulation of these PAH's with time. Application of benzo(a)anthracene, chrysene or benzo(a)pyrene in excess of background concentrations, or 0.1 to 0.2 $mg \cdot kg^{-1}$ (0.2 to 0.4 $kg \cdot ha^{-1}$) in soil, is likely to result in accumulation with time.

PACE recommends periodic monitoring of soil, sludge and groundwater on the basis that "it is important not only in tracing the overall impacts of landspreading but also in determining when sludge reapplications can be made and how successful certain management practices are in achieving the goal of rapid, environmentally safe hydrocarbon degradation and contaminant demobilization" (PACE, 1981). **It is desirable that selected PAH's be added to the list of parameters to be analyzed, with analysis of sludge to be performed when it is applied and periodic analysis of soil depending on the concentration in sludge.**

Conditions in soil on a land treatment site are not optimal for disappearance mechanisms throughout the year. Biological and chemical degradation may be retarded by cold temperatures or anaerobic conditions. Thus, rates of disappearance in field situations may be lower or higher than those observed in this incubation study. Chemical and biological degradation and volatilization may be enhanced by the practice of regularly cultivating the soil used for land treatment. Cultivation would increase air exchange, thus enhancing aerobic reactions and volatilization and expose more surface area to the effects of photochemical reactions. Proper cultivation techniques also increase infiltration of rainwater. This would minimize surface water contamination by runoff, but it is not known how it would affect the movement of waste material through the soil profile. Because of their tendency to be bound to solids it is unlikely that PAH's would move through soil in solution. They may, however, move in association with mobile organic colloids. Practices such as cultivation and fertilization may also encourage breakdown of organic material releasing bound PAH's and making them more susceptible to removal

mechanisms. At this time, however, little is known about the significance of soil bound PAH's in the environment. **Based on the results of this study, it appears that regular cultivation would enhance the major mechanisms which remove PAH's from soil.** Operators of land treatment sites should be aware of the concentrations of PAH's in wastes disposed to land and the persistence of PAH's in soil on their sites.

Studies using specific industrial wastes added to soil are required to adequately assess the persistence of PAH in those wastes when applied to land. These studies require adequate replication (minimum of triplicate samples) and should proceed until the concentration of target compounds approach background concentrations to ensure an adequate estimation of persistence in soil. Such studies to investigate the persistence of PAH in oil refinery waste applied to soil have been initiated at the Wastewater Technology Centre with the support of PACE.

Based on the results of this study, the following recommendations are made:

1. That those responsible for the disposal of oily wastes to land be made aware that certain PAH's could persist and accumulate in soil. It is desirable that they institute waste and soil monitoring programs to assess accumulation on individual sites.

2. That long term studies be performed to assess the environmental impact, including effect on groundwater quality and bioavailability, of PAH's added to different soil and waste types and under a variety of environmental conditions.

3. That the identity and fate of soil bound PAH's and metabolites should be determined.

4. That studies be undertaken to identify waste management techniques which enhance PAH degradation or removal from soil.

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GLOSSARY

Bioaccumulation - uptake and retention of a chemical by an organism resulting with time in a concentration in the organism higher than that in its surrounding environment, and possibly resulting in an increase in concentration in the organism, or in others that feed on it.

Chemical analogue - a chemical which can take the place of another in a reaction.

Cooxidation - oxidation of two substrates, one of which is usually recalcitrant, in which the more easily oxidized substrate provides a source of carbon and energy.

Cytotoxins - chemicals that are toxic to cells.

Depuration - removal as an impurity, excretion.

Dihydroxylation - addition of hydrogen to two oxygen groups on a molecule.

Dioxygenase - a degrading enzyme which catalyzes the incorporation of two oxygen atoms into a substrate molecule.

Gastrointestinal - related to the stomach and intestines.

Hepatobiliary - related to the liver and bile.

Hydrophobic - not readily wettable or soluble in water.

Lipophilic - readily soluble in a lipid (fat-like) substance, soluble in non-polar substances.

Microsomal - associated with microsomes, organelles which are responsible for oxidative metabolism (respiration) in cells.

Monooxygenase - a degrading enzyme which catalyzes the incorporation of one oxygen atom into a substrate molecule.

Reaction rate/ order - the kinetics of a reaction are often described in terms of a rate, a rate constant and an order. The rate of a reaction is described as the resulting change in concentration (of substrate or product) with time. On a normal graph of concentration vs time the rate of reaction is illustrated by the slope. This rate may be constant over time (linear slope) or may vary over time according to the mechanism of the reaction (non-linear slope). In this study the kinetics have not been defined on the basis of the actual mechanisms of PAH disappearance from soil, as the mechanisms are very complex and not well understood. The assumption has been made, however, that the rate of reaction is

proportional to the concentration of substrate raised to some power (rate = kC^n). The proportionality is defined by the rate constant (k) and the power (^{n}) of the concentration (C) is known as the reaction order. Thus, the rate constant and reaction order, together, define how the rate of the reaction will be affected by different concentrations of substrate. Because the concentration changes with time, as the reaction proceeds, the rate constant and reaction order define the change in rate of reaction with time. This is illustrated by the change in slope on a graph of concentration vs time. Although the mechanisms leading to a specific order of reaction have not been defined in this study, the order itself has been estimated by fitting the concentration data to the empirical model by non-linear regression.

Singlet oxygen - oxygen which has absorbed energy causing a change in electron spin orbitals to an antibonding or excited state.

Van der Waal's forces - a non-specific electrostatic interaction between atoms and molecules which arises from fluctuations in their electron distributions creating temporary dipoles. In soil the process causes adsorption of hydrocarbons onto the hydrophobic regions of soil constituents and is further reinforced by the fact that adsorption of hydrocarbons out of the water phase is thermodynamically favoured (Goring and Hamaker, 1972).

APPENDIX A

DEVELOPMENT OF A METHOD FOR THE ANALYSIS OF PAH'S IN A SOIL MATRIX

1 INTRODUCTION

This appendix provides detailed information regarding the development of a method for analysis of PAH's in soil. The existing methodology (Afghan and Wilkinson, unpublished report), which was developed for sediments, was studied for its applicability to soil samples of the type to be used in the degradation study. Modifications were also made to suit the existing apparatus in this laboratory. The method was assessed with respect to the following factors:

1. Reproducibility of response and retention times
2. Minimum detection limits
3. Recovery of PAH's from spiked water samples
4. Precision data on unspiked soil samples
5. Recovery from spiked soil samples.

The PAH's used in this study were the same as those being used in the degradation study. They consisted of naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene and benzo(a)pyrene. High Pressure Liquid Chromatography (HPLC) was chosen as the method of analysis for these types of samples because of its greater sensitivity, over Gas Chromatography, when coupled with suitable detectors.

2 METHOD DISCUSSION

The existing method developed by Afghan and Wilkinson (unpublished report) can be applied to sediments, effluents, fish tissues, precipitation samples, wastewaters, leachates, suspended solids and particulates. This method involves extraction with either a Polytron homogenizer using acetone or by Soxhlet with acetone:hexane (1:1). The resulting extract is then added to water and is partitioned into hexane by liquid-liquid extraction techniques. The hexane extract is then cleaned up using Gel Permeation Chromatography (GPC) and micro alumina columns. The resulting "cleaned" PAH fraction can then be analyzed using isocratic reverse phase HPLC techniques incorporating Ultra Violet and Fluorescence detectors for quantification.

For this study Afghan's method was modified and shortened slightly due to the nature of the samples and the apparatus available. The major differences in the method are as follows:

1. The extraction technique chosen was the use of the Polytron homogenizer. This technique requires less extraction time per sample compared to an individual Soxhlet system, but still yields excellent recoveries from spiked samples (see results and discussion).

2. Clean up of samples was limited to GPC. The soil samples being analyzed were not contaminated with large quantities of interfering substances and the quantities present were easily removed by GPC. This technique, however, incorporated a special GPC column obtained from Waters Scientific. The column consisted of a 1.2 meter x 2.5 cm stainless steel tube packed with 35 micron styragel stationary phase.

Because of the large size of this column and its increased capacity over standard GPC columns it was possible to clean up the whole extract with one injection. However, due to a large column void volume, this step is the slowest in the overall analysis requiring one and one-half hour per sample. This time can be reduced to fifty minutes per sample by careful injection of successive samples while the previous one is still on the column. This procedure worked well for the soil samples of the degradation study because of their simple elution profiles. However it will not necessarily work for all types of soil samples.

3. HPLC analysis conditions were changed from Afghan's method to enable better separation of the eight PAH's of interest. The conditions used incorporate the same isocratic mobile phase of acetonitrile:water (82:18) but the flow rate was reduced to 0.7 mL/min and the column was a Vydac 201 TP O.D.S. These conditions allowed for a complete separation of all eight PAH's even benzo(a)anthracene from chrysene. This simplified identification and quantitation.

As this method was to be used for a degradation study of PAH's with time it was necessary that conditions and sample treatment be constant from the first to the last sample. Because of this, the HPLC calibration was based on the initial spike levels of the soil samples of 5.0 $mg \cdot kg^{-1}$. When an 8 g sample is used, extraction and subsequent dilutions will yield a solution of 2.0 $mg \cdot L^{-1}$ of each PAH assuming 100% extraction efficiency. Actual extraction efficiency is close to this value as can be seen in the results from the recovery from spiked soils (results and discussion).

A 2.0 $mg \cdot L^{-1}$ standard of each PAH was then used to determine the optimum detector settings such that each of the signals was within the acceptable range of response. These settings were then maintained for the tests in this study as well as for all the degradation study samples. The settings for each detector were:

1. Kratos SF769 U.V. detector
   λ = 254 nm
   range = 0.02 Aufs (absorbance units, full scale)

2. Schoeffel FS970 Fluorometer
   λ excitation = 284 nm
   λ emission = 319 nm sharp cut filter
   range = 0.2 mA
   sensitivity = 4.8

An outline of the total procedure follows.

3 METHOD FOR THE ANALYSIS OF PAH'S IN A SOIL MATRIX

3.1 APPLICATION

This method was designed to identify and quantify eight PAH's in a soil matrix. These are naphthalene, phenanthrene, anthracene,

fluoranthene, pyrene, benzo(a)anthracene, chrysene and benzo(a)pyrene.
The method could be easily modified to determine other PAH's by changing flow rates and/or mobile phase compositions.

3.2 SAFETY PRECAUTION

2.1 Certain PAH's are known to be carcinogenic others are suspected, therefore these compounds must be handled with extreme caution. Contact with skin or clothing must be avoided. Use of disposable solvent resistant gloves during all handling of PAH standards or samples is recommended.

2.2 Sample extraction and preparation should be done in a fume hood or well ventilated area. All solvents and liquid wastes containing PAH should be disposed in an approved manner.

3.3 APPARATUS

Extraction and Concentration
- Polytron homogenizer with PT-10 generator
- 40 mL centrifuge tubes
- 2 litre separatory funnels
- 500 mL round bottom flasks
- Buchii rotoevaporator and temperature controlled bath
- 15 mL graduated centrifuge tube
- Whatman #41 filter papers, solvent washed prior to use

Gel Permeation Clean-up
- Rheodyne injection system or equivalent
- 2 mL sample injection loop
- 2 mL gas tight syringe
- High pressure liquid chromatographic pump with flow rates of 0.1 to 10.0 mL per minute
- Fluorescence detector (280 nm excitation and 389 s.c. emission filters)
- 35 micron styragel, 1.2 meters x 2.5 cm stainless steel column from Waters Scientific
- 250 mL round bottom flask
- 15 mL graduated centrifuge tubes
- Vortex evaporator

Analytical HPLC System
- Rheodyne injection system
- 10 µL sample injection loop
- 10 µL syringe
- Vydac 201 TP 54.6 ODS 5 µm column or equivalent
- Spectra Physics 8700 solvent delivery system or equivalent
- Kratos SF 769 variable wavelength U.V. detector or equivalent
- Schoeffel SF 970 LC fluorometer or equivalent (280 nm excitation and 389 S.C. emission filters)
- SP 4270 data collection and integration system or equivalent

3.4 REAGENTS

Solvents - Acetone (Caledon, DIG)
- Hexane (Caledon, DIG)
- Water (Organosorb* or equivalent)
- Chloroform (Caledon, HPLC)
- Acetonitrile (Caledon, HPLC)

PAH Standards
- All standards should be 98+% purity if possible, obtainable from Aldrich or Eastman
- 1000 $\mu g \cdot mL^{-1}$ stock solutions of each PAH in acetonitrile stored, sealed, in the dark at approx. 4°C

Sodium sulfate (BDH reagent) heated to 180°C for 48 hours and stored in a dessicator.
Saturated solution of sodium sulfate in organosorb water.

NOTE: Ensure all glassware is clean and has been thoroughly rinsed with acetone prior to use.

3.5 EXTRACTION AND CONCENTRATION

A(a) Weigh a portion of soil sample (1.00-2.00 g) and allow to dry to determine moisture content.
(b) Weigh 8.00 g of moist soil into a 40 mL (tared) centrifuge tube. Add 2 mL of water.
(c) Extract the soil with 20 mL of acetone using the Polytron homogenizer at medium speed for 2 minutes.
(d) Centrifuge the contents in a clinical centrifuge for 5 minutes at 2000 rpm. Decant the supernatant into a 2 L separatory funnel.
(e) Repeat (c) and (d) twice more and collect the supernatants in the same separatory funnel.

B(a) Add 1.5 liters of organosorb water to the above separatory funnel and 100 mL of saturated sodium sulfate solution (volume can vary depending on the extent of the emulsion).
(b) Add 75 mL of hexane and extract the solution for one to two minutes.
(c) Transfer the aqueous portion to another 2 L separatory funnel.
(d) Filter the remaining hexane fraction through sodium sulfate into a 500 mL round bottom flask.
(e) Rinse the funnel with 10 mL of hexane and pass this into the same flask.
(f) Repeat steps (b) to (e) above twice more and collect the hexane fractions in the same flask.

C(a) Concentrate the combined extracts on the rotoevaporator to 1 to 2 mL then transfer this with hexane washes to a 15 mL centrifuge tube.
(b) Evaporate the extracts to 1 mL under a stream of N_2.
(c) Add 5 mL of chloroform and reduce to 1 mL then rinse the sides of the centrifuge tube with chloroform and make up to a final volume of 5 mL. (Volume may vary depending on analyte concentrations).

*Organosorb: distilled water passed through carbon and ion exchange resins.

3.6 GPC CLEAN-UP

(a) Set-up the GPC system with a flow of 4.0 mL•minute^{-1} using chloroform as the mobile phase.

(b) Calibrate the system and obtain elution profiles using standard solutions of low molecular weight PAH (naphthalene) and high molecular weight PAH (benzo(a)pyrene). From these profiles the suitable fraction cut can be determined to obtain 95% recovery of all PAH's. (The high and low molecular weight PAH may vary depending on the PAH's being evaluated).

(c) Inject 1 mL of concentrate from 5.3 (c) above onto the calibrated system. Collect the appropriate fraction in a suitable size flask.

(d) The amount injected and the flow rate used may need to be varied to obtain a good separation.

(e) Repeat step (c) above until all of the sample has been processed.

(f) Rotoevaporate the combined fractions to 2 mL and transfer this to a centrifuge tube. Reduce the volume to 1 mL under a stream of N_2. Add 5 mL of acetonitrile and reduce the volume to 1 mL. Then make up to an appropriate volume with 50% acetonitrile in water. Usually a volume of 2 to 4 mL is appropriate to provide a sufficient concentration of analyte for HPLC analysis.

NOTE: Step (e) can be eliminated if the concentration of the analyte is sufficiently large (i.e., >5.0 µg•g^{-1} in the original sample).

3.7 HPLC ANALYSIS

(a) Set up the HPLC system under the following conditions:
Flow rate = 0.7 mL/min
Mobile Phase = 82% Acetonitrile in organosorb water
Detectors (1) U.V. at 254 nm
(2) Fluorescence excitation at 280 nm
emission at 389 sharp cut

(b) The instrument should be calibrated daily using a working standard of all 8 PAH's prepared from the stock solutions. The standard should be made in 1:1 acetonitrile:water.

(c) Flow rates and mobile phase composition may need to be varied to ensure proper separation of the PAH's, if other instruments or columns are being used or if PAH's other than the 8 discussed are to be chomatographed.

3.8 CALCULATION FOR THE PRESENCE OF PAH

$$\text{Conc.} = \frac{\text{Area 1} \times \text{Vol. 1} \times \text{Vol. 2} \times \text{Std}}{\text{Area 2} \times \text{Dry Weight} \times \text{Vol. 3}}$$

Area 1: Area under the peak of unknown concentration
Area 2: Area under the peak of known standard
Vol. 1: Volume of extract before GPC cleanup (mL)
Vol. 2: Final volume of extracts before HPLC analysis (mL)
Vol. 3: Volume of extract injected onto GPC column (mL)
Std: Concentration of standard (µg•g^{-1})

$$\text{Dry weight of sample} = \frac{\text{Wet weight} \times (100 - \% \text{ moisture})}{100}$$

4 RESULTS AND DISCUSSION

To determine the validity of this method for its use in soil sample analysis the procedure was evaluated as outlined below. The HPLC and GPC systems were calibrated daily with a standard mixture of the eight PAH's. The following are the tests and their results:

4.1 Reproducibility of response and retention time

Six replicate injections of a solution 2 $mg \cdot L^{-1}$ each of eight PAH's were injected onto the analytical column under the specified conditions. The first injection was used as the standard against which each subsequent injection was compared.

The results for the reproducibility of response and retention time are listed in Tables A4.1.1 and A4.1.2, respectively. A sample U.V. chromatogram of the eight PAH's is shown in Figure A4.1 As indicated, both tests showed the method gave reproducible results in both areas. Percent relative standard deviations ranged from 0.8% to 6.3% for response and from 0.1% to 0.4% for retention times. Day to day reproducibility of these numbers is enhanced by keeping the mobile phase flowing continually through the column and detectors.

4.2 Detection limit study

The detection limits were determined for both detectors at their most sensitive ranges:

a) U.V. range = 0.01 amps
   λ = 254 nm
b) Fluorescence range = 0.01 mA
   sensitivity = 5.0
   λ excit = 289 λ emission = 389 nm S.C.

This was accomplished by injecting successively lower concentrations of a standard mixture of the eight PAH's onto the HPLC column. The limit of detection was determined when the signal to noise ratio became 5:1.

The detection limits for each of the PAH's varied greatly as indicated in Table A4.2. This is to be expected as the response for each PAH differs for the specific excitation wavelength. As these limits apply only to the instruments themselves the actual detection limits for samples will depend more on background levels already present in the soil matrix.

4.3 Recovery from spiked water samples

Ten 1.5 liter samples of organosorb water were each spiked with a mixture of the eight PAH's. The spike consisted of a 10 $mg \cdot L^{-1}$ solution of each of the PAH's in acetonitrile. Three mLs of this spike were added to each sample and these were then extracted and analyzed as per the method.

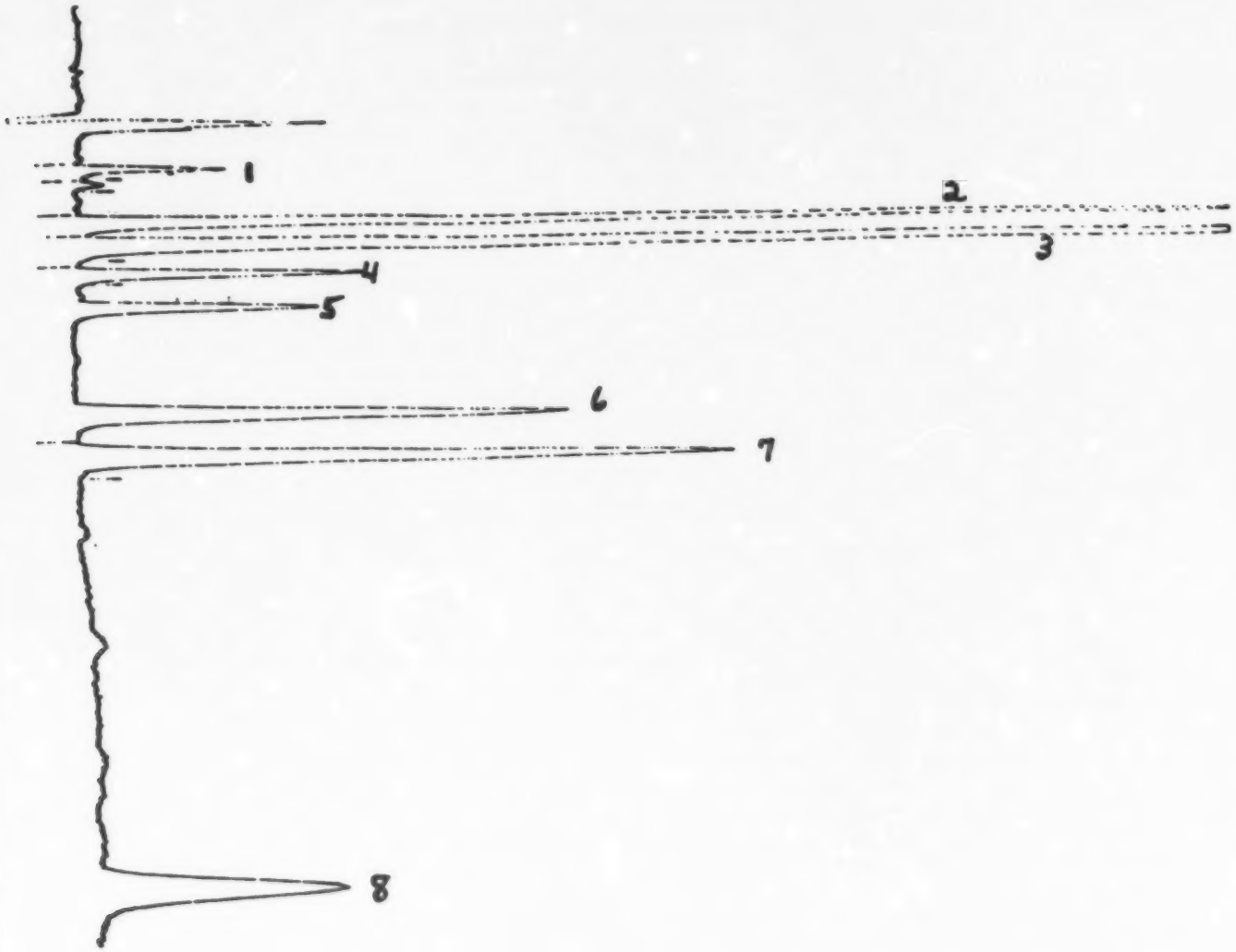
TABLE A4.1.1 REPRODUCIBILITY OF RESPONSE FOR 8 PAH'S

PAH	Injection $mg \cdot L^{-1}$ 1 Calibration	2	3	4	5	6	Mean	(∓)Std Dev	% Rel Std Dev
Naphthalene	2.00	2.00	2.00	2.00	2.02	2.03	2.01	1.3×10^{-2}	0.7
Phenanthrene	2.00	2.04	2.08	2.06	2.06	2.06	2.05	2.7×10^{-2}	1.3
Anthracene	2.00	2.02	2.06	2.04	2.03	2.04	2.03	2.0×10^{-2}	1.0
Fluoranthene	2.00	2.05	2.18	2.12	2.17	2.11	2.11	6.9×10^{-2}	3.3
Pyrene	2.00	2.13	2.28	2.28	2.34	2.37	2.23	1.4×10^{-1}	6.3
Benzo(a)Anthracene	2.00	2.05	2.17	2.10	2.13	2.17	2.10	6.8×10^{-2}	3.2
Chrysene	2.00	2.01	2.19	2.08	2.07	2.13	2.08	7.2×10^{-2}	3.5
Benzo(a)Pyrene	2.00	2.05	2.21	2.12	2.14	2.18	2.12	7.9×10^{-2}	3.7

TABLE A4.1.2. REPRODUCIBILITY OF RETENTION TIMES FOR 8 PAH'S

PAH	Injection $\mu g \cdot mL^{-1}$ 1 Calibration	2	3	4	5	6 (Minutes)	Mean	(∓)Std Dev	% Rel Std Dev
Naphthalene	5.52	5.52	5.52	5.53	5.52	5.53	5.52	5.2×10^{-3}	9.4×10^{-2}
Phenanthrene	7.18	7.18	7.19	7.19	7.20	7.19	7.19	7.5×10^{-3}	1.0×10^{-1}
Anthracene	7.87	7.87	7.88	7.88	7.89	7.87	7.88	8.2×10^{-3}	1.0×10^{-1}
Fluoranthene	8.73	8.74	8.76	8.75	8.76	8.73	8.75	1.4×10^{-2}	1.6×10^{-1}
Pyrene	9.82	9.82	9.85	9.83	9.85	9.82	9.83	1.5×10^{-2}	1.5×10^{-1}
Benzo(a)Anthracene	13.15	13.18	13.21	13.17	13.18	13.13	13.17	2.8×10^{-2}	2.1×10^{-1}
Chrysene	14.53	14.57	14.60	14.55	14.57	14.50	14.55	3.5×10^{-2}	2.4×10^{-1}
Benzo(a)Pyrene	28.02	28.13	28.08	27.97	28.17	27.89	28.04	1.0×10^{-1}	3.7×10^{-1}

FIGURE A4.1. HPLC CHROMATOGRAM OF PAH'S WITH U.V. DETECTOR; λ = 254 nm, 0.02 Aufs
CH_3CN/H_2O (82:18); FLOW = 0.7 mL·min^{-1}



(1) Naphthalene
(2) Phenanthrene
(3) Anthracene
(4) Fluoranthene
(5) Pyrene
(6) Benzo(a)anthracene
(7) Chrysene
(8) Benzo(a)pyrene

Results are outlined in Table 4.3. The recoveries for all PAH's range from 77% to 103%. The lower result for naphthalene, 77%, is probably due to its volatility and subsequent loss during evaporation.

4.4 Precision study based on unspiked soil samples

A sample of soil to be used in the degradation study was used as a blank. The sample was homogenized by shaking and stirring and a 2 g portion was analyzed to determine the moisture content. Ten samples were then prepared from the remaining soil and processed to determine the precision of the method at low level PAH concentrations.

Table 4.4 lists the results of the unspiked soil samples. Some of the PAH's are present consistently but others are in only a few of the samples. This is most likely due to a lack of homogeneity in the original portion of soil. The background levels are significantly lower, by 2 to 3 orders of magnitude in most cases compared to the spiked samples, and would not have a significant effect on the degradation study samples. Caution must be exercised when measuring at the lower levels; control has to be analyzed and the background subtracted. Relative standard deviations for these results were very large ranging from 37% to 300%. The higher standard deviations are associated with samples having very low concentrations.

4.5 Recovery of PAH's from spiked soil

A sample of the Donnybrook sandy loam used previously was obtained, homogenized and the moisture content determined. It was then spiked with a solution of eight PAH's in acetonitrile to yield a 10.0 $mg \cdot kg^{-1}$ spike based on the dry weight of the soil. The spiking process involved forming a slurry with the soil and acetonitrile solution, then allowing the solvent to evaporate, not quite to dryness. The dry weight was redetermined and the soil divided into four samples. Another portion of the same soil was treated in a similar manner to yield a spike of 10.3 µg/g in each PAH based on the soil's dry weight. From this, six samples were prepared. All 10 samples were then analyzed by the method to determine recovery from a soil matrix.

As shown in Table 4.5, mean recoveries ranged from 15% to 99%. These results are from 3% to 15% lower than the recoveries from water except for naphthalene which had only 15% total recovery from the soil. Again this low recovery for naphthalene is due to its volatility and probable loss during the spiking process. The low recoveries of the other PAH's are probably due to adsorption on the soil matrix. Percent relative standard deviations for these results range from 6% to 8% for the larger PAH's and 28% for naphthalene.

TABLE A4.2. MINIMUM DETECTION LIMITS FOR THE VARIOUS PAH'S BASED ON 10 µL SAMPLE LOOP

PAH	Detector	
	U.V. 254 n, 0.01 Aufs (pg injected)	Fluorescence 280 nm excitation 319 S.C. emission (pg injected)
Naphthalene	500	2000
Phenanthrene	30	200
Anthracene	30	200
Fluoranthene	200	1
Pyrene	300	1
Benzo(a)anthracene	200	0.5
Chrysene	100	20
Benzo(a)pyrene	400	0.5

TABLE 4.3 RECOVERIES FROM SPIKED WATER SAMPLES BASED ON 30 µg OF EACH PAH IN 1.5 L OF H_2O

PAH	Sample/% Recovery of Spike											
	1	2	3	4	5	6	7	8	9	10	Mean	±Std Dev
Naphthalene	90	96	84	71	77	93	68	60	72	60	77	4.0
Phenanthrene	97	108	91	94	93	97	97	90	99	89	96	5.5
Anthracene	88	91	78	80	82	83	75	79	82	78	92	4.8
Fluoranthene	103	100	102	101	104	108	103	101	101	109	108	3.0
Pyrene	96	101	94	99	101	106	102	103	105	104	101	3.8
Benzo(a)Anthracene	98	98	93	96	97	100	97	96	101	97	97	2.2
Chrysene	99	99	94	98	95	99	97	94	100	96	97	2.2
Benzo(a)Pyrene	88	86	77	85	83	86	75	83	87	88	83	4.6

TABLE 4.4 PRECISION DATA FOR UNSPIKED SOIL SAMPLES

PAH	Sample/pg·g^{-1} 1	2	3	4	5	6	7	8*	9	10	Mean	∓Std Dev
Naphthalene	--	--	--	--	--	--	--		--	--	--	--
Phenanthrene	178	112	238	115	124	79	214		188	104	150	55
Anthracene	9	18	--	35	6	--	81		--	--	17	27
Fluoranthene	--	--	125	--	--	--	134		--	--	29	57
Pyrene	--	--	--	--	--	--	180		--	--	20	60
Benzo(a)Anthracene	39	47	69	60	--	47	197		94	58	68	55
Chrysene	--	--	--	--	--	--	105		--	--	13	371
Benzo(a)Pyrene	113	97	144	105	83	136	314		182	104	142	71

-- Means none detected
* Sample 8 was destroyed while processing

TABLE 4.5 RECOVERY FROM SPIKED SOIL SAMPLES

PAH	Sample*/% recovery of spike 1	2	3	4	5	6	7	8	9	10	Mean	±Std Dev
Naphthalene	11	11	19	20	9	12	19	14	21	16	15	4.2
Phenanthrene	81	74	80	76	65	81	81	79	79	77	77	4.9
Anthracene	79	73	79	77	67	73	82	79	80	77	77	4.4
Fluoranthene	92	84	89	85	81	97	100	94	100	98	92	6.5
Pyrene	106	96	103	98	85	100	107	97	101	100	99	6.0
Benzo(a)Anthracene	95	85	93	87	82	98	105	94	98	97	94	6.8
Chrysene	98	87	93	87	82	96	102	92	99	94	93	6.3
Benzo(a)Pyrene	80	72	75	75	72	86	90	83	85	82	80	6.1

* 1st four from spike level = 10.0 $\mu g \cdot g^{-1}$
last six from spike level = 10.3 $\mu g \cdot g^{-1}$

Overall, however, the recoveries and precision obtained with this method are considered to be satisfactory for the types of samples to be analyzed in the degradation study at the levels of spike (5 and 50 $\mu g \cdot g^{-1}$) being applied.

5 CONCLUSIONS AND RECOMMENDATIONS

This method has been shown to be suitable for the analysis of PAH's in soil samples. Naphthalene poses a problem due to its volatility and samples suspected to contain naphthalene should be kept sealed and frozen before being analyzed.

This method has potential for analysis of sludges as well as soils containing sludges. However further work and evaluations should be done before undertaking sludge analysis.

APPENDIX B

METHOD FOR ANALYSIS OF ^{14}C LABELLED PAH'S IN COMPONENTS OF THE BIOMETER FLASK

1 INTRODUCTION

Polynuclear aromatic hydrocarbons (PAH's) labelled with ^{14}C were degraded in soil placed in biometer flasks as described in Section 7.2 of the main body of the report. The parent compounds and their degradation products were expected to be found in three matrices:

- soil
- polyurethane plug
- NaOH absorber

This appendix will describe in detail the methods used for analysis of these matrices and will assess the effect of extraction method on measurement of soil extractability and irreversible sorption for PAH's.

The ^{14}C compounds from the soil and polyurethane plugs were extracted, concentrated and counted by liquid scintillation counting. The extract was treated by Thin Layer Chromatography (TLC) to separate the extracted radioactivity into parent compound and degradation product. Radioactivity absorbed into NaOH was separated by hexane extraction into inorganic ($^{14}CO_2$) and organic degradation products. Irreversibly bound (unextractable) radioactivity on the extracted soil and polyurethane plugs was measured by combustion of the samples to $^{14}CO_2$ which was trapped and counted.

2 METHOD DISCUSSION

2.1 Soil Extraction

Two methods of soil extraction were evaluated:

- Polytron extraction with acetone as described in Appendix A. Data obtained by this method were reported in Part II of the study to be consistent with Part I. In addition, the polytron method calls for a H_2O partitioning which allows the measurement of polar, water soluble metabolites.
- Soxhlet extraction with 1:1 Hexane:Acetone for comparison of extraction efficiency.

2.2 Fractionation of ^{14}C Dissolved in NaOH

To assess the fractionation of ^{14}C as CO_2 in NaOH, $Na_2{}^{14}CO_3$ was dissolved in H_2O and extracted with hexane. No radioactivity was transferred from H_2O to the hexane. When NaOH from the biometer flasks was extracted with hexane, radioactivity was sometimes extracted indicating the presence of organic material. This extraction was routinely carried out to distinguish between inorganic and organic degradation products trapped in the NaOH absorbing solution.

2.3 Thin Layer Chromatography

Thin layer chromatography (TLC) was used to indicate whether extracted radioactive material was the parent PAH or a degradation product. Since the identity of the degradation products was unknown it was

difficult to specify chromatographic conditions to separate these chemicals. It is likely that any biological attack on an aromatic ring would produce products with different chromatographic properties from the parent compound, and that these products would not be unsubstituted PAH's. It is unlikely that all degradation products would chromatograph identically to parent PAH in two different solvent systems. The procedure adopted was to chromatograph the concentrated extracts on silica gel TLC plates in Hexane:Acetone:Toluene (1:1:1) which gives R_f factors for PAH's of 0.9 and in Hexane which gives R_f values of 0.3 for PAH's. It was not necessary to separate different PAH's since only one parent compound at a time was used as tracer.

The TLC plate was the Whatman LK5DF silica gel pate. It was convenient for routine use since it has a preabsorbent area for sample application and is divided into lanes for multiple sample use.

A detailed description of the method follows.

3 METHOD FOR THE ANALYSIS OF PAH'S IN COMPONENTS OF THE BIOMETER FLASK

3.1 Materials

3.1.1 Radiochemical

All ^{14}C labelled chemicals used as tracers were obtained from California Bionuclear Corp., Sun Valley, California. Napthalene (1-^{14}C, CBN564), Benzo(a)Pyrene (7,10-^{14}C, CBN 114) and Anthracene (9-^{14}C, CBN 086) were purchased at 98% radiochemical purity and were used as received. The labelled compounds were dissolved in toluene for application to the biometer flask soil.

3.1.2 Instrumental

All samples were counted for ^{14}C on a LKB model 1217 Liquid Scintillation counter. The External Standard Ratio (ESR) method of automatic window setting and the ESR method of quench correction for dpm calculation was used. Chemiluminescence was routinely monitored by the instrument.

Combustion analysis of the samples was carried out in a Biological Material Oxidizer (BMO/R.J. Harvey Instrument Corp.).

3.1.3 Standard and Reagents

All samples were counted in 22 mL plastic vials with 10 mL Amersham PCS liquid scintillation cocktail. Standards were prepared by dissolving one LKB-wallac ^{14}C-O Internal Standard pellet (^{14}C=105400 dpm) in 10 mL PCS and adding various volumes of blank sample to the PCS. Several standards formed the quench calibration curve and were stored in the instrument memory for automatic dpm calculation. Reagents were DIG, acetone, toluene, hexane, methanol (Caledon Laboratories), reagent grade

KOH, acetic acid (Fisher Scientific), mannitol (BDH). The liquid scintillator was PCS (Amersham). The CO_2 trapping solution was Oxisorb CO_2 (New England Nuclear) mixed 1:2 with PCS.

3.2 Methods

3.2.1 Soil

(a) Extraction

Soxhlet

A 10 g soil sample was weighed into a soxhlet extraction tube and extracted overnight with 300 mL of 1:1 hexane:acetone. The recovered solvent was evaporated to 15 mL on a rotovap. One mL was taken for ^{14}C counting, 50 µL for TLC and the residue reserved for combustion analysis.

Polytron

A 10 g soil sample was weighed into a 40 mL centrifuge tube. To the tube was added 25 mL acetone and the contents extracted for 2 minutes with a polytron homogenizer. The tube was centrifuged for 5 minutes in a clinical centrifuge at 2000 rpm and the supernatant decanted. The sample was extracted twice more with acetone and the extracts combined. The volume of recovered acetone was measured and 1 mL taken for ^{14}C counting. The soil residue was reserved for combustion analysis. The recovered acetone, 50 mL H_2O and 50 mL hexane were added to a 1 L separatory funnel and extracted for 2 minutes. The aqueous phase was extracted with two more 50 mL portions of hexane. The combined hexane extracts were evaporated to 15 mL. A 1 mL sample of the hexane concentrate and a 5 mL sample of extracted H_2O were taken for ^{14}C counting.

(b) Thin Layer Chromatography (TLC)

Using a microliter syringe, 50 µL of the soxhlet extract was applied to the preadsorbent area of each of 2 TLC plates (Whatman LK5DF silica gel, 250 µ thickness, 20 x 20 cm plate). Several other samples and a standard were applied to alternating lanes of the TLC plate. The plates were developed in ascending fashion, one plate in 1:1:1 hexane:acetone: toluene, the other plate in hexane to a height of 12-14 cm.

After drying, the lanes were divided into 1 cm sections and the silica gel recovered. The recovery of the silica gel layer was carried out by connecting the small end of a disposable pipet to a vacuum pump and suctioning into the pipet a small amount of glass wool. With the vacuum still applied each silica gel section was mechanically dislodged and suctioned into its own disposable pipet. The vacuum pump was then disconnected and 3x1 mL of toluene passed through the pipet to elute the labelled compounds in to a scintillation vial. Scintillation cocktail was then added and the samples were counted for ^{14}C.

3.2.2 Residue Analysis

After removal of solvent by air-drying, a sample of 0.05-0.08 g of extracted soil was burned at 900°C in a stream of O_2 in a Biological Material Oxidizer. After catalytic oxidation of combustion gases the CO_2 produced was trapped in a solution of 2+1 PCS + Oxisorb CO_2 (New England Nuclear). The trapping solution was counted for ^{14}C.

3.2.3 Polyurethane Plugs

The entire polyurethane plug was extracted in a soxhlet apparatus, concentrated and counted as per the soil method. This sample could not be extracted by the polytron method.

3.2.4 NaOH

Two 1 mL samples of 0.1 N NaOH were removed by syringe from each biometer flask. One sample was neutralized with 1 mL 0.1 N acetic acid and counted directly for total ^{14}C. The other sample was shaken for 2 minutes with 4 mL hexane in a test tube to extract organic ^{14}C. After phase separation the hexane was counted, and the extracted NaOH was neutralized with 1 mL 0.1N acetic acid and counted.

3.3 Statistical Assessment of Soil Extraction Methods

Soil samples taken for ^{14}C analysis in Part II of the PAH study (described in the main body of the report) were extracted using both the soxhlet and polytron methods. This analysis allowed statistical assessment of the extraction efficiency of the procedures, with all the treatments used in the Part II study, by means of a replicated 2^4 factorial design. The factors included in the factorial analysis are identified in Table B-1. Analysis of variance was used to assess significant differences against Mean Square Error at the 99% confidence level. Although many of these comparisons have been discussed in Section 8.2 for the polytron data alone, the experiment provided a unique opportunity to assess the effects of extraction method on extractability and irreversible sorption of the PAH's.

Table B-1

Factors Varied to Assess Extraction Efficiency

Factor	Level A	Level B
Extraction	Soxhlet	Polytron
PAH	Benzo(a)Pyrene	Anthracene
Concentration	50 µg/g	5 µg/g
Sterility	$HgCl_2$ added	no $HgCl_2$ added

4 RESULTS AND DISCUSSION

Table B-2 presents the results of statistical analysis for the percentages of ^{14}C found in the extract and soil residue with two extraction procedures (polytron and soxhlet), two PAH's (benzo(a)pyrene and anthracene), two concentrations of each PAH (5 and 50 $mg \cdot kg^{-1}$) and two "sterility" treatments (with and without $HgCl_2$ treatment). The effects of interactions between these treatments are also presented in the table and are indicated by combinations of the first letter of the treatments involved (for example, EP indicates the interaction of extraction method on the PAH effect). Mean squares (a measure of variance) and F values (a comparison of mean squares to the error variance) are provided for each comparison. An F value greater than 7.5 indicates a variance significantly greater than experimental error, therefore a significant treatment effect, with a confidence level of 99%.

Table B-2
Analysis of Variance Table for Extraction and Soil Residue

Source of Variation	Extract		Soil Residue	
	Mean Square	F	Mean Square	F
Extraction (E)	6.034	17.44*	4.078	74.15*
PAH (P)	1.626	4.70	0.239	4.34
Concentration (C)	2.813	8.13*	7.829	142.35*
Sterility (S)	0.046	0.13	0.863	15.69*
EP	0.057	0.16	0.192	3.49
EC	0.509	1.47	0.242	4.40
ES	0.316	0.91	0.076	1.38
PC	0.929	2.68	0.733	13.33*
PS	0.439	1.27	0.009	0.16
CS	0.166	0.48	0.058	1.05
EPC	0.123	0.36	0.029	0.53
EPS	0.036	0.10	0.159	2.89
ECS	0.005	0.01	0.077	1.40
PCS	0.209	0.60	0.406	7.38
EPCS	0.246	0.71	0.034	0.62
Error	0.346		0.055	

Degrees of Freedom: Total = 47
Error = 32
Others = 1

F(1, 32, .01) = 7.50

* Significant Effect at 99% level

4.1 Extraction into Solvent

PAH recovery in the extract ranged from 36.1 to 87.4%. A significant main effect was observed for the extraction method. The average recovery by the soxhlet procedure (74.5%) was higher than that by the polytron procedure (62.8%). Average values for all significant effects are presented in Table B-3. No other effects of extraction procedure on the percentage of extractable PAH were noted.

A significant main effect was noted for PAH concentration, which likely resulted from reactions occurring in soil during the incubation period, as discussed in Section 8.2. The results indicated that, despite an overall reduction in extraction efficiency with the polytron method, the increase in extractable PAH with the high application rate was consistent for both extraction procedures and for both PAH's (i.e., the EC and EP interactions were not significant). At the 50 $\mu g \cdot g^{-1}$ application level 72.6% of activity was recovered as opposed to 64.6% at the 5 $\mu g \cdot g^{-1}$ level.

4.2 Soil Residue by Combustion

After overnight soxhlet treatment with 1:1 hexane:acetone or three successive polytron extractions with acetone, 2.2% - 16.1% of the added radioactivity was found associated with the solids. Average values for significant effects are presented in Table B-3.

The main effect of extraction procedure was significant. Polytron and soxhlet extraction left an average of 7.4% and 4.5% of ^{14}C, respectively, attached to soil. This effect is consistent with the greater extraction efficiency noted with the extract data for the soxhlet technique. As observed with the extract data, no other effects of extraction procedure were significant.

The effects of PAH concentration and soil sterilization were significant, as discussed in Section 8.2. These effects were the same, however, regardless of extraction procedure. The difference due to soil sterilization was not reflected in the extract results, probably because the difference was small relative to the magnitude and variance of the soil extract values.

There was a significant interaction between PAH and the concentration of PAH. The difference between the percentage of benzo(a)pyrene bound at the low concentration (average 9.3%) and the high concentration (average 3.8%) was greater than the difference observed for anthracene (7.0% at the low concentration and 4.2% at the high concentration). This effect was consistant for both extraction procedures although the mechanism for this effect is not clear. The effect of PAH is compounded with differences in experimental conditions such as the length of incubation time. Although the main effect of PAH was not significantly different, the significant interaction term between PAH and concentration could have been caused by differences in experimental conditions.

TABLE B-3
Table of Means for Significant Effects

	Extract	
Extraction	Soxhlet	74.5%
	Polytron	62.8%
Concentration	50 µg/g level	72.6%
	5 µg/g level	64.6%
	Soil Residue	
Extraction	Soxhlet	4.5%
	Polytron	7.4%
Concentration	50 µg/g level	4.0%
	5 µg/g level	8.1%
Sterility	Sterile (Hg)	6.6%
	Unsterile	5.2%
PAH	Anth at 5	7.0%
Concentration	Anth at 50	4.2%
Interaction	BAP at 5	9.3%
	BAP at 50	3.8%

5 CONCLUSIONS

The polytron extraction procedure permitted fractionation of extractable polar and nonpolar ^{14}C labelled chemicals and combustion of non-extractable (soil bound) ^{14}C. There was some loss of extraction efficiency, however, in comparison to the soxhlet procedure. The loss in extraction efficiency did not have a significant effect on the evaluation of treatment effects in this study. The polytron procedure therefore was considered useful in identifying the mechanisms affecting PAH's in soil.

The use of ^{14}C labelling, with fractionation into extractable and volatile, polar and nonpolar chemicals and soil bound chemicals, was useful in following the fate of PAH's in soil. The procedures, however, did not result in the identification of soil bound ^{14}C or of metabolites in general. Additional methods development in those areas would be beneficial.